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(54) Title: FATTY ACID ELONGASES

(57) Abstract

Nucleic acids are disclosed that encode fatty acid β-keto acyl synthases from plants. Such synthases are effective for producig very long chain fatty acids (VLCFA), e.g., C22 to C26, preferentially saturated but also monounsaturated. Also disclosed are polypeptides encoded by such nucleic acids. Transgenic plants expressing these polypeptides exhibit altered levels of VLCFA in one or more tissues, such as seeds or leaves.

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FATTY ACID ELONGASES

Field of the Invention

This invention relates to fatty acid elongase complexes and nucleic acids encoding elongase proteins. More particularly, the invention relates to nucleic acids encoding ß-keto acyl synthase proteins that are effective for producing very long chain fatty acids, polypeptides produced from such nucleic acids and transgenic plants expressing such nucleic acids.

Background of the Invention

Plants are known to synthesize very long chain fatty acids (VLCFAs). VLCFAs are saturated or unsaturated monocarboxylic acids with an unbranched even-numbered carbon chain that is greater than 18 carbons in length. Many VLCFAs are 20-32 carbons in length, but VLCFAs can be up to 60 carbons in length. Important VLCFAs include erucic acid (22:1, i.e., a 22 carbon chain with one double bond), nervonic acid (24:1), behenic acid (22:0), and arachidic acid (20:0).

Plant seeds accumulate mostly 16- and 18-carbon fatty acids. VLCFAs are not desirable in edible oils. Oilseeds of the *Crucifereae* (e.g., rapeseed) and a few other plants, however, accumulate C20 and C22 fatty acids (FAs). Although plant breeders have developed rapeseed lines that have low levels of VLCFAs for edible oil purposes, even lower levels would be desirable. On the other hand, vegetable oils having elevated levels of VLCFAs are desirable for certain industrial uses, including uses as lubricants, fuels and as a feedstock for plastics, pharmaceuticals and cosmetics.

The biosynthesis of saturated fatty acids up to an 18-carbon chain occurs in the chloroplast. C2 units from acyl thioesters are linked sequentially, beginning with the condensation of acetyl Coenzyme A (CoA) and malonyl acyl carrier protein (ACP) to form a C4 acyl fatty acid. This condensation reaction is catalyzed by a ß-ketoacyl synthase III (KASIII). ß-ketoacyl moieties are also referred to as 3-ketoacyl moieties.

The enzyme ß-ketoacyl synthase I (KASI) is involved in the addition of C2 groups to form the C6 to C16 saturated fatty acids. KASI catalyzes the stepwise condensation of a fatty acyl moiety (C4 to C14) with malonyl-ACP to produce a 3-ketoacyl-ACP product that is 2 carbons longer than the substrate. The last condensation reaction in the chloroplast, converting C16 to C18, is catalyzed by ß-ketoacyl synthase II (KASII).

Each elongation cycle involves three additional enzymatic steps in addition to the condensation reaction as discussed above. Briefly, the ß-ketoacyl condensation product is reduced to ß-hydroxyacyl-ACP, dehydrated to the enoyl-ACP, and finally reduced to a fully reduced acyl-ACP. The fully reduced fatty acyl-ACP reaction product then serves as the substrate for the next cycle of elongation.

The C18 saturated fatty acid (stearic acid, 18:0) can be transported out of the chloroplast and converted to the monounsaturate C18:1 (oleic acid), and the polyunsaturates C18:2 (linoleic acid) and C18:3 (α-linolenic acid). C18:0 and C18:1 can also be elongated outside the chloroplast to form VLCFAs. The formation of VLCFAs involves the sequential condensation of two carbon groups from malonyl CoA with a C18:0 or C18:1 fatty acid substrate. Elongation of fatty acids longer than 18 carbons depends on the activity of a fatty acid elongase complex to carry out four separate enzyme reactions

similar to those described above for fatty acid synthesis in the chloroplast. Fehling, Biochem. Biophys. Acta 1082:239-246 (1991). In plants, elongase complexes are distinct from fatty acid synthases since elongases are extraplastidial and membrane bound.

Mutations have been identified in an *Arabidopsis* gene associated with fatty acid elongation. This gene, designated the *FAE1* gene, is involved in the condensation step of an elongation cycle. See, WO 96/13582, incorporated herein by reference. Plants carrying a mutation in *FAE1* have significant decreases in the levels of VLCFAs in seeds. Genes associated with wax biosynthesis in jojoba have also been cloned and sequenced (WO 95/15387, incorporated herein by reference).

Very long chain fatty acids are key components of many biologically important compounds in animals, plants, and microorganisms. For example, in animals, the VLCFA arachidonic acid is a precursor to many prostaglandins. In plants VLCFAs are major constituents of triacylglycerols in many seed oils, are essential precursors for cuticular wax production, and are utilized in the synthesis of glycosylceramides, an important component of the plasma membrane.

Obtaining detailed information on the biochemistry of KAS enzymes has been hampered by the difficulties encountered when purifying membrane bound enzymes. Although elongase activities have been partially purified from a number of sources, or studied using cell fractions, the elucidation of the biochemistry of elongase complexes has been hampered by the complexity of the membrane fractions used as the enzyme source. For example, until recently, it was unclear as to whether plant elongase complexes were composed of a multifunctional polypeptide similar to the FAS found in

animals and yeast, or if the complexes existed as discrete and dissociable enzymes similar to the FAS of plants and bacteria. Partial purification of an elongase KAS, immunoblot identification of the hydroxy acyl dehydrase, and the recent cloning of a KAS gene (*FAE1*) suggest that the enzyme activities of elongase complexes exist on individual enzymes.

Summary of the Invention

The invention disclosed herein relates to an isolated polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above sequences that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

Also disclosed herein is an isolated polypeptide that has an amino acid sequence substantially identical to one of the following: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. Also disclosed are isolated polynucleotides encoding polypeptides substantially identical in their amino acid sequence to: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

The invention also relates to a transgenic plant containing a nucleic acid construct. The nucleic acid construct comprises a polynucleotide described above. The construct further comprises a regulatory element

operably linked to the polynucleotide. The regulatory element may a tissue-specific promoter, for example, an epidermal cell-specific promoter or a seed-specific promoter. The regulatory element may be operably linked to the polynucleotide in sense or antisense orientation. The plant has altered levels of very long chain fatty acids in tissues where the polynucleotide is expressed, compared to a parental plant lacking the nucleic acid construct.

A method is disclosed for altering the levels of very long chain fatty acids in a plant. The method comprises the steps of creating a nucleic acid construct and introducing the construct into the plant. The construct includes a polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. The polynucleotide is effective for altering the levels of very long chain fatty acids in the plant.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Figure 1 shows the time course of *in vitro* VLCFA synthesis by *FAE1* expressed in yeast, with 3 different acyl-CoA substrates.

Figure 2 shows the rates of *in vitro* VLCFA synthesis and the VLCFA profiles of *FAE1* expressed in yeast, with 3 different acyl-CoA substrates.

Figure 3 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL1 polynucleotide (SEQ ID NO:1).

Figure 4 shows the deduced amino acid sequence (SEQ ID NO:2) for the EL1 coding sequence of Figure 3.

Figure 5 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL2 polynucleotide (SEQ ID NO:3).

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:4) for the EL2 coding sequence of Figure 5.

Figure 7 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL3 polynucleotide (SEQ ID NO:5).

Figure 8 shows the deduced amino acid sequence (SEQ ID NO:6) for the EL3 coding sequence of Figure 7.

Figure 9 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL4 polynucleotide (SEQ ID NO:7).

Figure 10 shows the deduced amino acid sequence (SEQ ID NO:8) for the EL4 coding sequence of Figure 9.

Figure 11 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL5 polynucleotide (SEQ ID NO:9).

Figure 12 shows the deduced amino acid sequence (SEQ ID NO:10) for the EL5 coding sequence of Figure 11.

Figure 13 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL6 polynucleotide (SEQ ID NO:11).

Figure 14 shows the deduced amino acid sequence (SEQ ID NO:12) for the EL6 coding sequence of Figure 13.

Figure 15 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL7 polynucleotide (SEQ ID NO:13).

Figure 16 shows the deduced amino acid sequence (SEQ ID NO:14) for the EL7 coding sequence of Figure 15.

Description of the Preferred Embodiments

The present invention comprises isolated nucleic acids (polynucleotides) that encode polypeptides having ß-ketoacyl synthase activity. The novel polynucleotides and polypeptides of the invention are involved in the synthesis of very long chain fatty acids and are useful for modulating the total amounts of such fatty acids and the specific VLCFA profile in plants.

A polynucleotide of the invention may be in the form of RNA or in the form of DNA, including cDNA, synthetic DNA or genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded, can be either the coding strand or non-coding strand. An RNA analog may be, for example, mRNA or a combination of ribo- and deoxyribonucleotides. Illustrative examples of a polynucleotide of the invention are shown in Figs. 3, 5, 7, 9, 11, 13 and 15.

A polynucleotide of the invention typically is at least 15 nucleotides (or base pairs, bp) in length. In some embodiments, a polynucleotide is about 20 to 100 nucleotides in length, or about 100 to 500 nucleotides in length. In other embodiments, a polynucleotide is greater than about 1500 nucleotides in length and encodes a polypeptide having the amino acid sequence shown in Figs. 4, 6, 8, 10, 12, 14 or 16.

In some embodiments, a polynucleotide of the invention encodes analogs or derivatives of a polypeptide having the deduced amino acid sequence of Figs. 4, 6, 8,

10, 12, 14 or 16. Such fragments, analogs on derivatives include, for example, naturally occurring allelic variants, non-naturally occurring allelic variants, deletion variants and insertion variants, that do not substantially alter the function of the polypeptide.

A polynucleotide of the invention may further comprise additional nucleic acids. For example, a nucleic acid fragment encoding a secretory or leading amino acid sequence can be fused in-frame to the amino terminal end of one of the EL1 through EL7 polypeptides. Other nucleic acid fragments are known in the art that encode amino acid sequences useful for fusing in-frame to the KAS polypeptides disclosed herein. See, e.g., U.S. 5,629,193 incorporated herein by reference. A polynucleotide may further comprise one or more regulatory elements operably linked to a KAS polynucleotide disclosed herein.

The present invention also comprises polynucleotides that hybridize to a KAS polynucleotide disclosed herein. Such a polynucleotide typically is at least 15 nucleotides in length. Hybridization typically involves Southern analysis (Southern blotting), a method by which the presence of DNA sequences in a target nucleic acid mixture are identified by hybridization to a labeled oligonucleotide or DNA fragment probe. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook et al., (1989) *Molecular Cloning*, second edition, Cold Spring Harbor Laboratory, Plainview; NY.

A polynucleotide can hybridize under moderate stringency conditions or, preferably, under high

stringency conditions to a KAS polynucleotide disclosed herein. High stringency conditions are used to identify nucleic acids that have a high degree of homology to the probe. High stringency conditions can include the use of low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC); 0.1% sodium lauryl sulfate (SDS) at 65°C. Alternatively, a denaturing agent such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is the use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Moderate stringency conditions refers to hybridization conditions used to identify nucleic acids that have a lower degree of identity to the probe than do nucleic acids identified under high stringency conditions. Moderate stringency conditions can include the use of higher ionic strength and/or lower temperatures for washing of the hybridization membrane, compared to the ionic strength and temperatures used for high stringency hybridization. For example, a wash solution comprising 0.060 M NaCl/0.0060 M sodium citrate (4X SSC) and 0.1% sodium lauryl sulfate (SDS) can be used at 50°C, with a last wash in 1X SSC, at 65°C. Alternatively, a hybridization wash in 1X SSC at 37°C can be used.

Hybridization can also be done by Northern analysis (Northern blotting), a method used to identify RNAs that hybridize to a known probe such as an

oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ^{32}P, by biotinylation or with an enzyme. The RNA to be analyzed can be usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., *supra*.

A polynucleotide has at least about 70% sequence identity, preferably at least about 80% sequence identity, more preferably at least about 90% sequence identity to SEQ ID NO:1, 3, 5, 7, 9, 11, or 13. Sequence identity can be determined, for example, by computer programs designed to perform single and multiple sequence alignments.

A polynucleotide of the invention can be obtained by chemical synthesis, isolation and cloning from plant genomic DNA or other means known to the art, including the use of PCR technology carried out using oligonucleotides corresponding to portions of SEQ ID NO:1, 3, 5, 7-9, 11 or 13. Polymerase chain reaction (PCR) refers to a procedure or technique in which target nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, incorporated herein by reference, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, and the

like. Alternately, a cDNA library (in an expression vector) can be screened with KAS-specific antibody prepared using peptide sequence(s) from hydrophilic regions of the KAS protein of SEQ ID NO:2 and technology known in the art.

A polypeptide of the invention comprises an isolated polypeptide having the deduced amino acid sequence of Figs. 2, 4, 6, 8, 10 and 12, as well as derivatives and analogs thereof. By "isolated" is meant a polypeptide that is expressed and produced in an environment other than the environment in which the polypeptide is naturally expressed and produced. For example, a plant polypeptide is isolated when expressed and produced in bacteria or fungi. Similarly, a plant polypeptide is isolated when its gene coding sequence is operably linked to a chimeric regulatory element and expressed in a tissue where the polypeptide is not naturally expressed. A polypeptide of the invention also comprises variants of the KAS polypeptides disclosed herein, as discussed above.

A full-length KAS coding sequence may comprise the sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. Alternatively, a chimeric full-length KAS coding sequence may be formed by linking, in-frame, nucleotides from the 5′ region of a first KAS gene to nucleotides from the 3′ region of a second KAS gene, thereby forming a chimeric KAS protein.

It should be appreciated that nucleic acid fragments having a nucleotide sequence other than the KAS sequences disclosed in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 will encode a polypeptide having the exemplified amino acid coding sequence of SEQ ID NO:2, 4, 6, 8, 10, 12 or 14, respectively. The degeneracy of the genetic code is well-known to the art; i.e., for many amino acids, there

is more than one nucleotide triplet which serves as the codon for the amino acid.

It should also be appreciated that certain amino acid substitutions can be made in protein sequences without affecting the function of the protein. Generally, conservative amino acid substitutions or substitutions of similar amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and Structure*, Vol. 5, Suppl. 3, pp. 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity.

A nucleic acid construct of the invention comprises a polynucleotide as disclosed herein linked to another, different polynucleotide. For example, a full-length KAS coding sequence may be operably fused in-frame to a nucleic acid fragment that encodes a leader sequence, secretory sequence or other additional amino acid sequences that amy be usefully linked to a polypeptide or peptide fragment.

A transgenic plant of the invention contains a nucleic acid construct as described herein. In some embodiments, a transgenic plant contains a nucleic acid construct that comprises a polynucleotide of the invention operably linked to at least one suitable regulatory sequence in sense orientation. Regulatory sequences typically do not themselves code for a gene product. Instead, regulatory sequences affect the expression level of the polynucleotide to which they are

linked. Examples of regulatory sequences are known in the art and include, without limitation, minimal promoters and promoters of genes preferentially or exclusively expressed in seeds or in epidermal cells of stems and leaves. Native regulatory sequences of the polynucleotides disclosed herein can be readily isolated by those skilled in the art and used in constructs of the invention. Other examples of suitable regulatory sequences include enhancers or enhancer-like elements, introns, 3' non-coding regions such as poly A sequences and other regulatory sequences discussed herein. Molecular biology techniques for preparing such chimeric genes are known in the art.

In other embodiments, a transgenic plant contains a nucleic acid construct comprising a partial or a full-length KAS coding sequence operably linked to at least one suitable regulatory sequence in antisense orientation. The chimeric gene can be introduced into a plant and transgenic progeny displaying expression of the antisense construct are identified.

One may use a polynucleotide disclosed herein for cosuppression as well as for antisense inhibition. Cosuppression of genes in plants may be achieved by expressing, in the sense orientation, the entire or partial coding sequence of a gene. See, e.g., WO 04\11516, incorporated herein by reference.

Transgenic techniques for use in the invention include, without limitation, *Agrobacterium*-mediated transformation, viral vector-mediated transformation electroporation and particle gun transformation. Illustrative examples of transformation techniques are described in U.S. Patent 5,204,253, (particle gun) and U.S. Patent 5,188,958 (*Agrobacterium*), incorporated herein by reference. Transformation methods utilizing the Ti and Ri plasmids of *Agrobacterium spp.* typically

use binary-type vectors. Walkerpeach, C. et al., in Plant Molecular Biology Manual, S. Gelvin and R. Schilperoort, eds., Kluwer Dordrecht, C1:1-19 (1994). If cell or tissue cultures are used as the recipient tissue for transformation, plants can be regenerated from transformed cultures by techniques known to those skilled in the art.

Techniques are known for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues. Monocots which have been successfully transformed and regenerated include wheat, corn, rye, rice, and asparagus. See, e.g., U.S. Patent Nos. 5,484,956 and 5,550,318, incorporated herein by reference.

For efficient production of transgenic plants from plant cells, it is desirable that the plant tissue used for transformation possess a high capacity for regeneration. Transgenic plants of woody species such as poplar and aspen have also been obtained. Technology is also available for the manipulation, transformation, and regeneration of gymnosperm plants. For example, U.S. Patent No. 5,122,466 describes the biolistic transformation of conifers, with preferred target tissue being meristematic and cotyledon and hypocotyl tissues. U.S. Patent No. 5,041,382 describes enrichment of conifer embryonal cells.

Seeds produced by a transgenic plant(s) can be grown and then selfed (or outcrossed and selfed) to obtain seeds homozygous for the construct. Seeds can be analyzed in order to identify those homozygotes having the desired expression of the construct. Transgenic plants may be entered into a breeding program, e.g., to introgress the novel construct into other lines, to transfer the construct to other species, or for further

selection of other desirable traits. Alternatively, transgenic plants may be propagated vegetatively for those species amenable to such techniques. A nucleic acid construct of the invention can alter the levels of very long chain fatty acids in plant tissues expressing the polynucleotide, compared to VLCFA levels in corresponding tissues from an otherwise identical plant not expressing the polynucleotide. A comparison can be made, for example, between a non-transgenic plant of a plant line and a transgenic plant of the same plant line. Levels of VLCFAs having 20-32 carbons and/or levels of VLCFAs having 32-60 carbons can be altered in plants disclosed herein. Plants having an altered VLCFA composition may be identified by techniques known to the skilled artisan, e.g., thin layer chromatography or gas-liquid chromatography (GLC) analysis of the appropriate plant tissue.

A suitable group of plants with which to practice the invention are the *Brassica* species, including *B. napus*, *B. rapa*, *B.juncea*, and *B. hirta*. Other suitable plants include, without limitation, soybean (*Glycine max*), sunflower (*Helianthus annuus*) and corn (*Zea mays*).

A method according to the invention comprises introducing a nucleic acid construct into a plant cell and producing a plant (as well as progeny of such a plant) from the transformed cell. Progeny includes descendants of a particular plant or plant line, e.g., seeds developed on an instant plant are descendants. Progeny of an instant plant include seeds formed on F_1, F_2, F_3, and subsequent generation plants, or seeds formed on BC_1, BC_2, BC_3, and subsequent generation plants.

Methods and compositions according to the invention are useful in that the resulting plants and plant lines have desirable alterations in very long chain fatty acid composition. Suitable tissues in which to

express polynucleotides and/or polypeptides of the invention include, without limitation, seeds, stems and leaves. Leaf tissues of interest include cells and tissues of the epidermis, e.g., cells that are involved in forming trichomes. Of particular interest are epidermal cells involved in forming the cuticular layer. The cuticular layer comprises various very long chain fatty acids and VLCFA derivatives such as alkanes, esters, alcohols and aldehydes. Altering the composition and amount of VLCFAs in epidermal cells and tissues may enhance defense mechanisms and drought tolerance of plants disclosed herein.

Polynucleotides of the invention can be used as markers in plant genetic mapping and plant breeding programs. Such markers may include RFLP, RAPD, or PCR markers, for example. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process. Marker-assisted breeding techniques may be used in addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence from a desired KAS that has been introduced into a plant line and is being crossed into other plant lines.

Plants and plant lines disclosed herein preferably have superior agronomic properties. Superior agronomic characteristics include, for example, increased seed germination percentage, increased seedling vigor, increased resistance to seedling fungal diseases (damping off, root rot and the like), increased yield, and improved standability.

While the invention is susceptible to various modifications and alternative forms, certain specific embodiments thereof are described in the general methods

and examples set forth below. It should be understood, however, that these examples are not intended to limit the invention to the particular forms disclosed but, instead the invention is to cover all modifications, equivalents and alternatives falling within the scope of the invention.

EXAMPLES

Example 1

Cloning and Expression of FAE1 in Yeast Cells

The open reading frame of the *Arabidopsis FAE1* gene was amplified directly by PCR, using *Arabidopsis thaliana cv.* Columbia genomic DNA as a template, pfu DNA polymerase and the following primers: 5'CTCGAGGAGCAATGACGTCCGTTAA-3' and 5'-CTCGAGTTAGGACCGACCGTTTTG-3'. The PCR product was blunt-end cloned into the *Eco* RV site of pBluescript (Stratagene, La Jolla, CA),

The *FAE1* gene was excised from the Bluescript vector with Bam*H*1, and then subcloned into the pYEUra3 (Clontech, Palo Alto, CA). pYEUra3 is a yeast centromere-containing, episomal plasmid that is propagated stably through cell division. The *FAE1* gene was inserted downstream of a GAL1 promoter in pYEUra3. The GAL1 promoter is induced when galactose is present in the medium and repressed when glucose is present in the growth medium.

Insertion of the *FAE1* gene in the sense orientation was confirmed by PCR, and pYEUra3/*FAE1* was used to transform *Saccharomyces cerevisiae* strain AB1380 using a lithium acetate procedure as described in Gietz, R. and Woods, R., in Molecular Genetics of Yeast: Practical Approaches, Oxford Press, pp. 121-134 (1994). Plasmid DNA was isolated from putative transformants, and

the presence of the *FAE1*/pYEUra3 construct was confirmed by Southern analysis.

Yeast transformed with pYEUra3 having *FAE1* operably linked to the GAL1 promoter were grown in the presence of galactose or glucose and were analyzed for the expression of *FAE1*. As a control, yeast transformed with pYEUra3 containing no insert were also assayed. Analysis of such control preparations yielded fatty acid compositions and fatty acid elongation rates similar to those of yeast transformed with pYEUra3/*FAE1* and grown with glucose as the carbon source.

The fatty acid composition of yeast cells grown in the presence of galactose was compared to that of cells grown in the presence of glucose, to determine if VLCFA were found in the galactose-induced cells.

Transformed yeast cells were grown overnight in YPD media at 30°C with vigorous shaking. One hundred μl of the overnight culture were used to inoculate 40 ml of complete minimal uracil dropout media (CM-Ura) supplemented with either glucose or galactose (2% w/v). Cultures were grown at 30°C to an OD_{600} of approximately 1.3 to 1.5. Cells were harvested by centrifugation at 5000 Xg for 10 min. Total lipids were extracted from the cells with 2 volumes of 4N KOH in 100% methanol for 60 min. at 80°C. Fatty acids were saponified and methyl esters were prepared by drying the samples and resuspending in 0.5 ml of boron trichloride in methanol (10% v/v). Samples were incubated at 50°C for 15 min in a sealed tube. About 2 ml of water was then added and the fatty methyl esters were extracted thrice with 1 ml of hexane. Extracts were dried under nitrogen and redissolved in hexane. See Hlousek-Radojcic, A. et al., Plant J. 8:803-809. Methyl esters were analyzed on an HP 5890 series II gas chromatograph equipped with a 5771MSD and 7673 auto injector (Hewlett-Packard, Cincinnati, OH).

Methyl esters were separated on a DB-23 (J&W Scientific) capillary column (30 m X 0.25 mm X 0.25 μm). The column was operated with helium carrier gas and splitless injection (injection temperature 280°C, detector temperature 280°C). After an initial 3 min. at 100°C, the oven temperature was raised to 250° at 20°C min^{-1} and maintained at that temperature for an additional 3 min. The identity of the peaks was verified by cochromatography with authentic standards and by mass spectrometer analysis.

The results clearly revealed the appearance of both 20:1 and 22:1 acyl-CoA products in galactose-induced yeast containing the *FAE1* coding sequence. Uninduced yeast cells failed to accumulated significant amounts of fatty acids longer than C18. These results indicate that expression of *FAE1* in yeast resulted in functional KAS activity and functional elongase activity.

Example 2

FAE1 Activity in Yeast Microsomes

The functional expression of the *FAE1* KAS was analyzed by isolating microsomes from transformed yeast cells and assaying these microsomes *in vitro* for elongase activity.

Transformed yeast cells were grown in the presence of either glucose or galactose (2% w/v) as described in Example 1. Cells were harvested by centrifugation at 5000 Xg for 10 min and washed with 10 ml ice cold isolation buffer (IB), which contains 80 mM Hepes-KOH, pH 7.2, 5 mM EGTA, 5 mM EDTA, 10 mM KCl, 320 mM sucrose and 2 mM DTT). Cells were then resuspended in enough IB to fill a 1.7 ml tube containing 700 μl of 0.5 μm glass beads and yeast microsomes were isolated from the cells essentially as described in Tillman, T. and Bell, R., J. Biol. Chem. 261:9144-9149 (1986). The microsomal

membrane pellet was recovered by centrifugation at 252,000 xg for 60 min. The pellet was rinsed by resuspending in 40 ml fresh IB and again recovered by centrifugation at 252000 Xg for 60 min. Microsomal pellets were resuspended in a minimal volume of IB, and the protein concentration adjusted to 2.5 $\mu g\ \mu l^{-1}$ by addition of IB containing 15% glycerol. Microsomes were frozen on dry ice and stored at -80°C. The protein concentration in microsomes was determined by the Bradford method (Bradford, 1976).

Fatty acid elongase activity was measured essentially as described in Hlousek-Radojcic, A. et al., Plant J. 8:803-809 (1995). Briefly, the standard elongation reaction mix contained 80 mM Hepes-KOH, pH 7.2, 20 mM $MgCl_2$, 500 μM NADPH, 1 mM ATP, 100 uM malonyl-CoA, 10 μM CoA-SH and 15 μM radioactive acyl-CoA substrate. The radiolabeled substrate was either [1-^{14}C]18:1-CoA (50 uCi μmol^{-1}), [1-^{14}C]18:0-CoA (55 uCi μmol^{-1}), or [1-^{14}C]16:0-CoA (54 uCi μmol^{-1}). The reaction was initiated by the addition of yeast microsomes (5 μg protein) and the mixture incubated at 30° C for the indicated period of time. The final reaction volume was 25 μl.

Methyl esters of the acyl-CoA elongation products were prepared as described in Example 1. Methyl esters were separated on reversed phase silica gel KC18 TLC plates (Whatman, 250 uM thick), quantified by phosphorimaging, and analyzed on by ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). The detection limit for each product is about 0.001 nanomoles per min. per mg microsomal protein, depending on the phosphorimage exposure time.

Results of representative *in vitro* elongation assays are shown in Figs. 1 and 2. The results indicate that microsomes from galactose-induced cells expressing

FAE1 catalyzed multiple cycles of elongation starting with either C16:0 acyl CoA, C18:0 acyl CoA, or C18:1 acyl-CoA as the substrate (Fig. 1). The 16:0 and 18:0 acyl-CoA substrates were elongated to C26:0 acyl-CoA. In contrast, the 18:1-CoA substrate was elongated primarily to C20:1, with only low levels of C22:1 acyl-CoA being produced. Occasionally, trace levels of C24:1 CoA were also observed. Although the chain length of the products from the 18:1 acyl-CoA substrate were less than the chain length from the saturated acyl-CoA substrates, the rate of elongation of oleoyl-CoA was about 2- and 3-fold higher than the rates of elongation of 16:0-CoA and 18:0-CoA, respectively.

The elongation activity observed in microsomes from uninduced cells indicated that there was a low level of endogenous elongase activity when 18:1-CoA or 18:0-CoA were used as substrates. There was substantial 16:0-CoA elongase activity (10.1 nmol mg protein^{-1} at 30 min) in microsomes from uninduced cells (Fig. 2). However, the major product of 16:0 elongation using uninduced microsomes was C18:0 acyl CoA, with only small amounts of products beyond this length. The elongation of the 16:0 acyl-CoA substrate presumably is due to an endogenous yeast elongase.

Elongation of 18:1 CoA by microsomes from induced cells occurred at a rate about 18-fold higher than in microsomes isolated from the uninduced cells (Fig. 2). With microsomes from induced yeast, synthesis of 20:0 CoA from the 16:0 CoA substrate, occurred at a rate similar to that seen when the substrate was 18:0 CoA (4.2 vs. 5.1 nmol mg protein^{-1}). The total rate of elongation of [^{14}C] 16:0-CoA by microsomes from induced cells (15.8 nmol mg protein^{-1} at 30 min.) was more than 50% higher than elongation of [^{14}C] 16:0-CoA by microsomes from uninduced cells, suggesting that the *FAE1* KAS utilized 16:0-CoA as

a substrate in addition to C18-C24 acyl-CoAs. The *FAE1* elongase KAS activity, i.e., the difference in the 16:0 elongation rates between microsomes from induced and uninduced cells, was 5.7 nmol mg protein^{-1}. The elongation rate with the 16:0 substrate thus was similar to the elongase activity of the *FAE1* elongase KAS with the 18:0 substrate.

These results indicate that *FAE1* KAS expressed in yeast could synthesize 3-ketoacyl-CoA *in vitro* and, in combination with yeast reductases and dehydrases, could form a functional VLCFA elongase complex. In addition, these results suggest that *FAE1* is membrane-bound in yeast cells.

Example 3

Cloning and Sequencing of *Arabidopsis* Elongase Genes

The sequence of a jojoba seed cDNA (see WO 93/10241 and WO 95/15387, incorporated herein by reference) was used to search the Arabidopsis expressed sequence tag (EST) database of the Arabidopsis Genome Stock Center (The Ohio State University, Columbus, Ohio). The BLAST computer program (National Institutes of Health, Bethesda, MD, USA) was used to perform the search. The search identified two ESTs (ATTS1282 and ATTS3218) that had a high degree of sequence identity with the jojoba sequence. The ATTS1282 and ATTS3218 ESTs appeared to be partial cDNA clones rather than full-length clones based on the length of the jojoba sequence.

A genomic DNA library from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda GEM11 vector (Promega, Madison, Wisconsin) and was obtained from Ron Davis, Stanford University, Stanford, CA. The library was hybridized with ATTS1282 and ATTS3218 as probes and 2 clones were identified for each EST. Phage DNA was isolated from each of the hybridizing clones, the genomic

insert was excised with the restriction enzyme Sac I and subcloned into the plasmid pBluescript (Stratagene, La Jolla, CA). One clone from the ATTS1282 hybridization was designated EL1 and one clone from the ATTS3218 hybridization was designated EL2.

A yeast expression library, containing cDNA from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda YES expression vector described in Elledge et al. (Elledge, S. et al., Proc. Natl. Acad. Sci USA 88:1731-1735 (1991) and was obtained from Ron Davis at Stanford University, Stanford, CA. The library was hybridized with a EL2 partial cDNA probe. A full-length EL2 cDNA was not identified. However, the probe did identify a full-length cDNA which was designated EL3.

A consensus sequence for the C-terminal region of EL1, EL2 and the jojoba cDNA polypeptides was identified by sequence alignment using DNA analysis programs from DNAStar, Madison, Wisconsin. This consensus sequence was used to search the *Arabidopsis* EST database again for ß-keto acyl synthase sequences. These searches identified four additional putative ß-keto acyl synthase ESTs, which were designated EL4 through EL7. EL4, EL5, EL6, and EL7 have homology to Genbank Accession Nos. T04345, T44939, T22193 and T76700, respectively.

The lambda YES cDNA expression library described above was hybridized with the EL1 and EL4-EL7 ESTs as probes. This screen identified full-length cDNAs for EL1, EL5 and EL6.

The lambda GEM11 genomic library was hybridized with the EL4 and EL7 ESTs as probes. This screen identified full-length genomic clones for EL4 and EL7. Phage DNA was isolated from each of the hybridizing clones and subcloned into pBluescript as described above.

The 7 EL clones were sequenced on both strands with regions of overlap for each sequence run. Sequencing was carried out with an ABI automated sequencer (Applied Biosystems, Inc., Foster City, California), following the manufacturer's instructions.

The nucleotide sequences for the coding regions of EL1-EL7 are shown in Figs. 3, 5, 7, 9, 11, 13 and 15, respectively. The deduced amino acid sequences for EL1-EL7 are shown in Figs. 4, 6, 8, 10, 12, 14 and 16, respectively, using the standard one-letter amino acid code. The EL1, EL2 and EL7 genomic clones appeared to lack introns. The EL4 genomic clone contained one intron near the 5' end of the coding region.

The nucleotide sequences of the 7 EL polynucleotides were compared to 5 DNA sequences present in Genbank. Genbank, National Center for Biotechnology Information, Bethesda, MD. Two of the 5 accessions were cloned from members of the Brassicaceae: the *Arabidopsis* FAE1 sequence (Accession U29142) and a *Brassica napus* sequence (Accession U50771). Three of the accessions were cloned from jojoba (*Simmondsia chinensis*): 2 wax biosynthesis genes (Accessions I14084 and I14085) and a jojoba KAS gene (Accession U37088). See also U.S. Patent 5,445,947, incorporated herein by reference.

Multiple alignment of the 12 sequences was carried out with a computer program sold under the trade name MEGALIGN Lasergene by DNAStar (Madison, Wisconsin). Alignments were done using the Clustal method with weighted residue weight table. The nucleotide sequence similarity index and percent divergence based on the multiple alignment algorithm is shown in Table 1. The nucleotide sequences of EL1-EL7 are distinguishable from the 5 DNA sequences obtained from Genbank.

The deduced amino acid sequences of the EL1-7 polypeptides were compared with the MEGALIGN program to

the deduced amino acid sequences of the same 5 Genbank clones, using the Clustal method with PAM250 residue weight table. The amino acid sequence similarity and percent divergence are shown in Table 2. The amino acid sequences of EL1-EL7 polypeptides are distinguishable from those of the Genbank sequences.

TABLE 1

Nucleotide sequence pair distances of EL1-EL7, using Clustal method with weighted residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12		
1		77.5	62.4	58.8	57.0	54.9	47.0	42.8	42.9	43.1	44.7	41.3	1	ARAFAE1 U29142
2	18.1		61.0	57.9	55.4	53.7	46.9	42.7	44.1	42.9	42.3	40.5	2	BNaFAE1 U50771
3	40.4	41.0		70.5	59.3	56.4	46.7	48.5	48.1	48.6	46.5	43.5	3	EL2
4	43.9	44.3	28.0		56.3	55.4	46.5	47.0	45.1	47.2	47.4	42.3	4	EL3
5	40.7	42.3	45.0	45.0		68.0	54.0	46.8	46.6	46.4	49.0	47.2	5	EL5
6	45.8	48.9	46.0	47.3	32.4		53.6	48.6	48.2	48.6	49.0	49.2	6	EL7
7	74.1	71.0	69.4	67.3	64.3	64.5		49.8	49.2	49.8	47.7	48.2	7	EL6
8	68.1	66.2	63.4	63.1	65.5	64.2	56.1		97.7	99.7	48.4	45.8	8	JOJOKCS U37088
9	67.0	65.4	63.7	64.6	64.6	64.1	56.6	1.1		95.9	47.6	44.8	9	JOKCS10 I14084
10	67.2	65.2	61.8	61.4	64.1	63.0	56.3	0.2	1.1		48.4	45.3	10	JOKCS11 I14085
11	88.6	85.8	81.0	77.0	77.4	82.4	83.1	71.1	71.1	69.9		48.3	11	EL1
12	95.7	90.4	95.4	91.5	84.5	82.8	91.9	73.4	73.8	73.3	59.9		12	EL4
	1	2	3	4	5	6	7	8	9	10	11	12		

TABLE 2

Amino acid sequence pair distances of EL1-EL7, using Clustal method with PAM250 residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12		
1		72.0	62.9	59.8	60.9	60.2	50.3	51.9	52.1	51.5	49.1	42.0	1	EL2
2	31.1		60.1	57.5	58.7	57.1	49.8	49.8	50.0	49.2	49.6	44.4	2	EL3
3	47.4	48.7		82.4	60.7	63.0	50.0	51.4	51.6	50.8	47.8	43.9	3	ATFAE1 U29142
4	51.8	52.8	17.9		60.2	61.0	49.2	50.3	50.5	49.7	46.5	42.4	4	BNFAE1 U50771
5	49.0	51.3	45.8	46.2		75.8	61.0	58.7	58.9	58.3	55.0	55.6	5	EL7
6	52.6	55.5	42.8	46.5	29.3		61.8	55.7	55.7	54.9	52.9	50.5	6	EL5
7	74.7	70.5	71.8	74.4	52.0	50.8		52.8	52.8	51.8	53.4	51.6	7	EL6
8	66.7	69.2	66.2	67.3	54.8	59.8	67.7		99.8	96.9	53.1	52.0	8	JOJKCS U37088
9	66.3	68.7	66.2	67.3	54.0	59.3	67.7	0.2		96.9	53.1	51.9	9	JKCS11 I14085
10	66.3	69.7	66.6	67.8	54.5	60.7	68.6	1.8	1.6		51.7	50.7	10	JKCS10 I14084
11	73.6	73.7	72.8	74.4	60.8	66.0	67.2	63.9	63.9	65.3		50.8	11	EL1
12	84.8	85.5	82.7	83.3	60.6	70.8	67.1	68.5	68.5	69.9	69.4		12	EL4
	1	2	3	4	5	6	7	8	9	10	11	12		

Example 4

Expression of EL1 and EL2 in Yeast

The open reading frames (ORFs) for the EL2, EL4 and EL7 clones were amplified by PCR. The EL2 ORF was cloned into λYES using the primers: CTCGAGCAAGTCCACTACCACGCA and CTCGAGCGAGTCAGAAGGAACAAA. The EL4 ORF was cloned into pYEUra3 using the primers: GATAATTTAGAGAGGCACAGGGT and GTCGACACAAGAATGGGTAGATCCAA. The EL7 ORF was cloned into pYEUra3 using the primers: CAGTTCCTCAAACGAAGCTA and GTCGACTTCTCAATGGACGGTGCCGGA. Amplified products were cloned into pYEUra3 under the control of, and 3′ to, the GAL1 promoter. The resulting plasmids were transformed into yeast as described in Example 1.

Yeast cultures containing full-length EL1 in λYES and full-length EL2 in pYEUra3 were grown in the presence of galactose or glucose as described in Example 2. Microsomes were then prepared from each of the cultures and fatty acid elongation assays were carried out as described in Example 2.

In the first experiment, microsomes were prepared from galactose-induced cultures of EL1, EL2 and FAE1, and incubated with either [1-^{14}C] 18:0 acyl-CoA or [1-^{14}C] 18:1 acyl-CoA as substrate. The amounts of various reaction products synthesized after 30 minutes (min) were determined as described in Example 2. The results when 18:0 acyl-CoA was the substrate are shown in Table 3. The results when 18:1 acyl-CoA was the substrate are shown in Table 4.

Table 3.
Elongation of 18:0-CoA by FAE1, EL1 and EL2 Genes Expressed in Yeast

	ß-Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
Acyl-CoA Product	Rate[1]	(%)	Rate	(%)	Rate	(%)
20:0	0.369	64.3	0.084	38.8	0.108	41.8
22:0	0.113	18.6	0.047	21.9	0.053	20.7
24:0	0.065	10.7	0.043	19.9	0.052	20.3
26:0	0.038	6.3	0.042	19.4	0.044	17.2
Total	0.585	100.0	0.216	100.0	0.258	100.0

[1] Nanomoles/minute/mg of microsomal protein

Table 4.
Elongation of 18:1-CoA by FAE1, EL1 and EL2 Genes Expressed in Yeast

	ß-Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
Acyl-CoA Product	Rate[1]	(%)	Rate	(%)	Rate	(%)
20:1	1.131	84.6	0.111	80.8	0.091	84.1
22:1	0.206	15.4	0.026	19.2	0.017	15.9
24:1	0.0	0.0	0.0	0.0	0.0	0.0
26:1	0.0	0.0	0.0	0.0	0.0	0.0
Total	1.337	100.0	0.137	100.0	0.108	100.0

[1] Nanomoles/minute/mg of microsomal protein

The results shown in Tables 3 and 4 indicate that the EL1 and EL2 gene products have ß-ketoacyl synthase (KAS) activity and that the KAS reaction product can be utilized to form VLCFAs. The specific activities of the 3 KAS enzymes cannot be compared, since the relative amount of the heterologous KAS protein in each microsomal preparation is not known. However, the proportions of various reaction products can be compared between FAE1, EL1 and EL2.

The data shown in Table 3 indicate that the EL1 and EL2 KAS activities result in a higher proportion of saturated VLCFAs than does the FAE1 KAS activity. These

results suggest that EL1 and EL2 encode novel gene products, because EL1 and EL2 have a greater preference for C22:0 and C24:0 acyl-CoA substrates than does *FAE1*.

A comparison of the relative elongation activity of FAE1 with 18:0 and 18:1 substrates (Tables 3 and 4) indicates that FAE1 is more active when 18:1 is the substrate than when 18:0 is the substrate. In contrast, the overall rate of product formation with EL1 is less when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). EL2 is also less active when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). These results support the conclusion that EL1 and EL2 encode novel gene products and suggest that EL1 and EL2 have a preference for saturated fatty acids as substrates, whereas the FAE1 gene product has a preference for monounsaturated fatty acids as substrates.

In a second experiment, microsomes were prepared from galactose-induced and from glucose-repressed yeast cultures containing EL1 or EL2 coding sequences. The microsomal preparations were incubated with either 18:0 acyl-CoA or 18:1 acyl-CoA as substrate and the fatty acid reaction products determined as described above. The results with the 18:0 substrate are shown in Table 5. The results with the 18:1 substrate are shown in Table 6.

Table 5.
Elongation of 18:0-CoA by EL1 and EL2
With and Without Induction of Gen Expression

	ß-Keto Acyl Synthase Gene							
	EL1				EL2			
Acyl	+Glucose		+Galactose		+Glucose		+Galactose	
CoA	Rate[1]	(%)	Rate	(%)	Rate	(%)	Rate	(%)
20:0	0.007	100.0	0.074	55.8	0.030	81.3	0.107	43.1
22:0	0.000	0.0	0.023	17.4	0.002	5.1	0.044	17.8
24:0	0.000	0.0	0.020	15.3	0.005	13.6	0.048	19.1
26:0	0.000	0.0	0.015	11.5	0.000	0.0	0.050	20.0
Total	0.007	100.0	0.133	100.0	0.037	100.0	0.249	100.0

[1] Nanomoles/minute/mg of microsomal protein

Table 6.
Elongation of 18:1-CoA by EL1 and EL2
With and Without Induction of Gene Expression

	ß-Keto Acyl Synthase Gene							
	EL1				EL2			
Acyl	+Glucose		+Galactose		+Glucose		+Galactose	
CoA	Rate[1]	(%)	Rate	(%)	Rate	(%)	Rate	(%)
20:1	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0
22:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
24:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
26:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
Total	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0

[1] Nanomoles/minute/mg of microsomal protein

The results in Table 5 show *in vitro* elongase activity for EL1 and EL2 under induced (galactose) and uninduced (glucose) conditions. The comparison indicates that induction with galactose results in a large increase in overall elongase activity when 18:0 acyl CoA is the substrate (about 19-fold and 7-fold for EL1 and EL2, respectively). In contrast, induction when 18:1 acyl CoA is the substrate results in only a small increase in elongase activity (about 1.3-fold and 2-fold for EL1 and El2, respectively), as shown in Table 6.

The results in Table 5 show that little or no VLCFA products are made by yeast microsomes under uninduced conditions. Upon induction of EL1 and EL2 gene

expression, however, significant quantities of C20:0, C22:0, C24:0 and C26:0 are made. The data in Tables 5 and 6 are consistent with the results in Tables 3 and 4, which indicated that EL1 and EL2 were more active with a saturated fatty acid substrate than with a monounsaturated substrate.

The data in Tables 5 and 6 are also consistent with the data in Tables 3 and 4 indicating that the EL1 and EL2 gene products are more active in converting C24:0 to C26:0 than is *FAE1*.

In a third experiment, microsomes from induced and uninduced cultures containing EL1 or EL2 were incubated in the absence of cofactors involved in the ß-ketoacyl condensation reaction. Cultures were induced and microsomes were prepared as described in Example 2. *In vitro* assays were carried out as described in Example 2, except that either ATP, CoASH or both were omitted from the enzyme reaction mixture. In addition, one reaction was carried out in a complete mixture having 0.01 mM of cerulenin (Sigma, St. Louis, MO). Cerulenin is an inhibitor of some condensing enzymes. The results are shown in Tables 7-9.

Tabl 7.
Effect of Cofactors on 18:0-CoA Elongation[1]

Gene	Expt[4]	+Glu[2]	+Gal[2]	-ATP[3]	-CoA[3]	-A&C[3]	+ Cer[3]
EL1	1	.037	.109	.095	.105	.119	.141
	2	N.D.	.090	.125	.093	.270	.176
EL2	1	.033	.112	.168	.127	.143	.238
	2	N.D.	.120	.178	.133	.195	.302

[1] Activity in nanomoles/minute/mg of microsomal protein.
[2] +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.
[3] Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.
[4] Experiment No.

Table 8.

Effect of Cofactors on Elongation Products of EL1[1]

Prod.	+Glu[2]	+Gal[2]	-ATP[3]	-CoA[3]	-A&C[3]	+Cer[3]
20:0	53.9	46.2	34.4	47.8	41.7	46.7
22:0	14.4	18.7	13.7	18.0	19.4	16.2
24:0	18.5	18.1	20.6	19.1	16.7	17.7
26:0	13.2	17.1	31.4	15.2	22.3	19.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1] Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.

[2] +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.

[3] Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

Table 9.

Effect of Cofactors on Elongation Products of EL2[1]

Prod.	+Glu[2]	+Gal[2]	-ATP[3]	-CoA[3]	-A&C[3]	+Cer[3]
20:0	54.5	47.1	34.1	45.3	38.0	41.8
22:0	17.1	19.1	16.4	19.2	15.9	16.1
24:0	5.8	19.4	20.8	19.9	18.4	20.4
26:0	22.6	14.5	28.9	15.8	27.8	21.8
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1] Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.
[2] +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.
[3] Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

The results in Table 7 indicate that omission of ATP and/or CoA from the incubation mixture does not have a significant effect on the overall amounts of VLCFAs synthesized by the *in vitro* KAS activity of EL1 or EL2. The results also show that cerulenin does not inhibit the KAS activity of EL1 or EL2. The data in Table 8 and 9 confirm that EL1 and EL2 KAS activity produces significant amounts of C24:0 and C26:0 acyl CoA products.

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features shown in other of the specific embodiments.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those

skilled in the art without deviating from the spirit and scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: CARGILL, INCORPORATED

(ii) TITLE OF THE INVENTION: FATTY ACID ELONGASES

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C., P.A.
(B) STREET: 60 South Sixth Street, Suite 3300
(C) CITY: Minneapolis
(D) STATE: MN
(E) COUNTRY: USA
(F) ZIP: 55402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/868,373
(B) FILING DATE: 03-JUN-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lundquist, Ronald C
(B) REGISTRATION NUMBER: 37,875
(C) REFERENCE/DOCKET NUMBER: 07039/064WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 612-335-5050
(B) TELEFAX: 612-288-9696
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATGGATCGAG AGAGATTAAC GGCGGAGATG GCGTTTCGAG ATTCATCATC GGCCGTTATA     60
AGAATTCGAA GACGTTTGCC GGATTTATTA ACGTCCGTTA AGCTCAAATA CGTGAAGCTT    120
GGACTTCACA ACTCTTGCAA CGTGACCACC ATTCTCTTCT TCTTAATTAT TCTTCCTTTA    180
ACCGGAACCG TGCTGGTTCA GCTAACCGGT CTAACGTTCG ATACGTTCTC TGAGCTTTGG    240
TCTAACCAGG CGGTTCAACT CGACACGGCG ACGAGACTTA CCTGCTTGGT TTTCCTCTCC    300
TTCGTTTTGA CCCTCTACGT GGCTAACCGG TCTAAACCGG TTTACCTAGT GGATTTCTCC    360
TGCTACAAAC CGGAAGACGA GCGTAAAATA TCAGTAGATT CGTTCTTGAC GATGACTGAG    420
GAAAATGGAT CATTCACCGA TGACACGGTT CAGTTCCAGC AAAGAATCTC GAACCGGGCC    480
```

```
GGTTTGGGAG ACGAGACGTA TCTGCCACGT GGCATAACTT CAACGCCCCC GAAGCTAAAT    540
ATGTCAGAGG CACGTGCCGA AGCTGAAGCC GTTATGTTTG GAGCCTTAGA TTCCCTCTTC    600
GAGAAAACCG GAATTAAACC GGCCGAAGTC GGAATCTTGA TAGTAAACTG CAGCTTATTC    660
AATCCGACGC CGTCTCTATC AGCGATGATC GTGAACCATT ACAAGATGAG AGAAGACATC    720
AAAAGTTACA ACCTCGGAGG AATGGGTTGC TCCGCCGGAT TAATCTCAAT CGATCTCGCT    780
AACAATCTCC TCAAAGCAAA CCCTAATTCT TACGCTGTCG TGGTAAGCAC GGAAAACATA    840
ACCCTAAACT GGTACTTCGG AAATGACCGG TCAATGCTCC TCTGCAACTG CATCTTCCGA    900
ATGGGCGGAG CTGCGATTCT CCTCTCTAAC CGCCGTCAAG ACCGGAAGAA GTCAAAGTAC    960
TCGCTGGTCA ACGTCGTTCG AACACATAAA GGATCAGACG ACAAGAACTA CAATTGCGTG   1020
TACCAGAAGG AAGACGAGAG AGGAACAATC GGTGTCTCTT TAGCTAGAGA GCTCATGTCT   1080
GTCGCCGGAG ACGCTCTGAA AACAAACATC ACGACTTTAG GACCGATGGT TCTTCCATTG   1140
TCAGAGCAGT TGATGTTCTT GATTTCCTTG GTCAAAAGGA AGATGTTCAA GTTAAAAGTT   1200
AAACCGTATA TTCCGGATTT CAAGCTAGCT TTCGAGCATT TCTGTATTCA CGCAGGAGGT   1260
AGAGCGGTTC TAGACGAAGT GCAGAAGAAT CTTGATCTCA AAGATTGGCA CATGGAACCT   1320
TCTAGAATGA CTTTGCACAG ATTTGGTAAC ACTTCGAGTA GCTCGCTTTG GTATGAGATG   1380
GCTTATACCG AAGCTAAGGG TCGGGTTAAA GCTGGTGACC GACTTTGGCA GATTGCGTTT   1440
GGATCGGGTT TCAAGTGTAA TAGTGCGGTT TGGAAAGCGT TACGACCGGT TTCGACGGAG   1500
GAGATGACCG GTAATGCTTG GGCTGGTTCG ATTGATCAAT ATCCGGTTAA AGTTGTGCAA   1560
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 520 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Arg Glu Arg Leu Thr Ala Glu Met Ala Phe Arg Asp Ser Ser
 1               5                  10                  15
Ser Ala Val Ile Arg Ile Arg Arg Arg Leu Pro Asp Leu Leu Thr Ser
            20                  25                  30
Val Lys Leu Lys Tyr Val Lys Leu Gly Leu His Asn Ser Cys Asn Val
        35                  40                  45
Thr Thr Ile Leu Phe Phe Leu Ile Ile Leu Pro Leu Thr Gly Thr Val
    50                  55                  60
Leu Val Gln Leu Thr Gly Leu Thr Phe Asp Thr Phe Ser Glu Leu Trp
65                  70                  75                  80
Ser Asn Gln Ala Val Gln Leu Asp Thr Ala Thr Arg Leu Thr Cys Leu
                85                  90                  95
Val Phe Leu Ser Phe Val Leu Thr Leu Tyr Val Ala Asn Arg Ser Lys
            100                 105                 110
Pro Val Tyr Leu Val Asp Phe Ser Cys Tyr Lys Pro Glu Asp Glu Arg
        115                 120                 125
Lys Ile Ser Val Asp Ser Phe Leu Thr Met Thr Glu Glu Asn Gly Ser
    130                 135                 140
Phe Thr Asp Asp Thr Val Gln Phe Gln Gln Arg Ile Ser Asn Arg Ala
145                 150                 155                 160
Gly Leu Gly Asp Glu Thr Tyr Leu Pro Arg Gly Ile Thr Ser Thr Pro
                165                 170                 175
Pro Lys Leu Asn Met Ser Glu Ala Arg Ala Glu Ala Glu Ala Val Met
            180                 185                 190
Phe Gly Ala Leu Asp Ser Leu Phe Glu Lys Thr Gly Ile Lys Pro Ala
        195                 200                 205
Glu Val Gly Ile Leu Ile Val Asn Cys Ser Leu Phe Asn Pro Thr Pro
    210                 215                 220
Ser Leu Ser Ala Met Ile Val Asn His Tyr Lys Met Arg Glu Asp Ile
225                 230                 235                 240
Lys Ser Tyr Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Leu Ile Ser
                245                 250                 255
Ile Asp Leu Ala Asn Asn Leu Leu Lys Ala Asn Pro Asn Ser Tyr Ala
            260                 265                 270
Val Val Val Ser Thr Glu Asn Ile Thr Leu Asn Trp Tyr Phe Gly Asn
        275                 280                 285
```

```
Asp Arg Ser Met Leu Leu Cys Asn Cys Ile Phe Arg Met Gly Gly Ala
    290                 295                 300
Ala Ile Leu Leu Ser Asn Arg Arg Gln Asp Arg Lys Lys Ser Lys Tyr
305                 310                 315                 320
Ser Leu Val Asn Val Val Arg Thr His Lys Gly Ser Asp Asp Lys Asn
                325                 330                 335
Tyr Asn Cys Val Tyr Gln Lys Glu Asp Glu Arg Gly Thr Ile Gly Val
            340                 345                 350
Ser Leu Ala Arg Glu Leu Met Ser Val Ala Gly Asp Ala Leu Lys Thr
        355                 360                 365
Asn Ile Thr Thr Leu Gly Pro Met Val Leu Pro Leu Ser Glu Gln Leu
    370                 375                 380
Met Phe Leu Ile Ser Leu Val Lys Arg Lys Met Phe Lys Leu Lys Val
385                 390                 395                 400
Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile
                405                 410                 415
His Ala Gly Gly Arg Ala Val Leu Asp Glu Val Gln Lys Asn Leu Asp
            420                 425                 430
Leu Lys Asp Trp His Met Glu Pro Ser Arg Met Thr Leu His Arg Phe
        435                 440                 445
Gly Asn Thr Ser Ser Ser Ser Leu Trp Tyr Glu Met Ala Tyr Thr Glu
    450                 455                 460
Ala Lys Gly Arg Val Lys Ala Gly Asp Arg Leu Trp Gln Ile Ala Phe
465                 470                 475                 480
Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Lys Ala Leu Arg Pro
                485                 490                 495
Val Ser Thr Glu Glu Met Thr Gly Asn Ala Trp Ala Gly Ser Ile Asp
            500                 505                 510
Gln Tyr Pro Val Lys Val Val Gln
        515                 520
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1479 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGGATTACC CCATGAAGAA GGTAAAAATC TTTTTCAACT ACCTCATGGC GCATCGCTTC      60
AAGCTCTGCT TCTTACCATT AATGGTTGCT ATAGCCGTGG AGGCGTCTCG TCTTTCCACA     120
CAAGATCTCC AAAACTTTTA CCTCTACTTA CAAAACAACC ACACATCTCT AACCATGTTC     180
TTCCTTTACC TCGCTCTCGG GTCGACTCTT TACCTCATGA CCCGGCCCAA ACCCGTTTAT     240
CTCGTTGACT TTAGCTGCTA CCTCCCACCG TCGCATCTCA AAGCCAGCAC CCAGAGGATC     300
ATGCAACACG TAAGGCTTGT ACGAGAAGCA GGCGCGTGGA AGCAAGAGTC CGATTACTTG     360
ATGGACTTCT GCGAGAAGAT TCTAGAACGT TCCGGTCTAG GCCAAGAGAC GTACGTACCC     420
GAAGGTCTTC AAACTTTGCC ACTACAACAG AATTTGGCTG TATCACGTAT AGAGACGGAG     480
GAAGTTATTA TTGGTGCGGT CGATAATCTG TTTCGCAACA CGGGAATAAG CCCTAGTGAT     540
ATAGGTATAT TGGTGGTGAA TTCAAGCACT TTTAATCCAA CACCTTCGCT ATCAAGTATC     600
TTAGTGAATA AGTTTAAACT TAGGGATAAT ATAAAGAGCT TGAATCTTGG TGGGATGGGG     660
TGTAGCGCTG GAGTCATCGC TATCGATGCG GCTAAGAGCT TGTTACAAGT TCATAGAAAC     720
ACTTATGCTC TTGTGGTGAG CACGGAGAAC ATCACTCAAA ACTTGTACAT GGGTAACAAC     780
AAATCAATGT TGGTTACAAA CTGTTTGTTC CGTATAGGTG GGGCCGCGAT TTTGCTTTCT     840
AACCGGTCTA TAGATCGTAA ACGCGCAAAA TACGAGCTTG TTCACACCGT GCGGGTCCAT     900
ACCGGAGCAG ATGACCGATC CTATGAATGT GCAACTCAAG AAGAGGATGA AGATGGCATA     960
GTTGGGGTTT CCTTGTCAAA GAATCTACCA ATGGTAGCTG CAAGAACCCT AAAGATCAAT    1020
ATCGCAACTT TGGGTCCGCT TGTTCTTCCC ATAAGCGAGA AGTTTCACTT CTTTGTGAGG    1080
TTCGTTAAAA AGAAGTTTCT CAACCCCAAG CTAAAGCATT ACATTCCGGA TTTCAAGCTC    1140
GCATTCGAGC ATTTCTGTAT CCATGCGGGT GGTAGAGCGC TAATTGATGA GATGGAGAAG    1200
AATCTTCATC TAACTCCACT AGACGTTGAG GCTTCAAGAA TGACATTACA CAGGTTTGGT    1260
AATACCTCTT CGAGCTCCAT TTGGTACGAG TTGGCTTACA CAGAAGCCAA AGGAAGGATG    1320
ACGAAAGGAG ATAGGATTTG GCAGATTGCG TTGGGGTCAG GTTTTAAGTG TAATAGTTCA    1380
```

GTTTGGGTGG CTCTTCGTAA CGTCAAGCCT TCTACTAATA ATCCTTGGGA ACAGTGTCTA 1440
CACAAATATC CAGTTGAGAT CGATATAGAT TTAAAAGAG 1479

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 493 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Asp Tyr Pro Met Lys Lys Val Lys Ile Phe Phe Asn Tyr Leu Met
 1               5                  10                  15
Ala His Arg Phe Lys Leu Cys Phe Leu Pro Leu Met Val Ala Ile Ala
            20                  25                  30
Val Glu Ala Ser Arg Leu Ser Thr Gln Asp Leu Gln Asn Phe Tyr Leu
        35                  40                  45
Tyr Leu Gln Asn Asn His Thr Ser Leu Thr Met Phe Phe Leu Tyr Leu
    50                  55                  60
Ala Leu Gly Ser Thr Leu Tyr Leu Met Thr Arg Pro Lys Pro Val Tyr
65                  70                  75                  80
Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Ala Ser
                85                  90                  95
Thr Gln Arg Ile Met Gln His Val Arg Leu Val Arg Glu Ala Gly Ala
            100                 105                 110
Trp Lys Gln Glu Ser Asp Tyr Leu Met Asp Phe Cys Glu Lys Ile Leu
        115                 120                 125
Glu Arg Ser Gly Leu Gly Gln Glu Thr Tyr Val Pro Glu Gly Leu Gln
    130                 135                 140
Thr Leu Pro Leu Gln Gln Asn Leu Ala Val Ser Arg Ile Glu Thr Glu
145                 150                 155                 160
Glu Val Ile Ile Gly Ala Val Asp Asn Leu Phe Arg Asn Thr Gly Ile
                165                 170                 175
Ser Pro Ser Asp Ile Gly Ile Leu Val Val Asn Ser Ser Thr Phe Asn
            180                 185                 190
Pro Thr Pro Ser Leu Ser Ser Ile Leu Val Asn Lys Phe Lys Leu Arg
        195                 200                 205
Asp Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly
    210                 215                 220
Val Ile Ala Ile Asp Ala Ala Lys Ser Leu Leu Gln Val His Arg Asn
225                 230                 235                 240
Thr Tyr Ala Leu Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr
                245                 250                 255
Met Gly Asn Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Ile
            260                 265                 270
Gly Gly Ala Ala Ile Leu Leu Ser Asn Arg Ser Ile Asp Arg Lys Arg
        275                 280                 285
Ala Lys Tyr Glu Leu Val His Thr Val Arg Val His Thr Gly Ala Asp
    290                 295                 300
Asp Arg Ser Tyr Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile
305                 310                 315                 320
Val Gly Val Ser Leu Ser Lys Asn Leu Pro Met Val Ala Ala Arg Thr
                325                 330                 335
Leu Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Ile Ser
            340                 345                 350
Glu Lys Phe His Phe Phe Val Arg Phe Val Lys Lys Lys Phe Leu Asn
        355                 360                 365
Pro Lys Leu Lys His Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His
    370                 375                 380
Phe Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Met Glu Lys
385                 390                 395                 400
Asn Leu His Leu Thr Pro Leu Asp Val Glu Ala Ser Arg Met Thr Leu
                405                 410                 415
```

```
His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala
            420                 425                 430
Tyr Thr Glu Ala Lys Gly Arg Met Thr Lys Gly Asp Arg Ile Trp Gln
        435                 440                 445
Ile Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ser Val Trp Val Ala
    450                 455                 460
Leu Arg Asn Val Lys Pro Ser Thr Asn Asn Pro Trp Glu Gln Cys Leu
465                 470                 475                 480
His Lys Tyr Pro Val Glu Ile Asp Ile Asp Leu Lys Glu
                485                 490
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1512 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CTACGTCAGG GTAGAACAAA GAGTAAACAC TTAAGCAAAA CAATTTGTCC TACTCTTAGG     60
TTATCTCCAA TGAAGAACTT AAAGATGGTT TTCTTCAAGA TCCTCTTTAT CTCTTTAATG    120
GCAGGATTAG CCATGAAAGG ATCTAAGATC AACGTAGAAG ATCTCCAAAA GTTCTCCCTC    180
CACCATACAC AGAACAACCT CCAAACCATA AGCCTTCTAT TGTTTCTTGT CGTTTTTGTG    240
TGGATCCTCT ACATGTTAAC CCGACCTAAA CCCGTTTACC TTGTTGATTT CTCCTGCTAC    300
CTTCCACCGT CGCATCTCAA GGTCAGTATC CAAACCCTAA TGGGACACGC AAGACGTGCA    360
AGAGAAGCAG GCATGTGTTG GAAGAACAAA GAGAGCGACC ATTTAGTTGA CTTCCAGGAG    420
AAGATTCTTG AACGTTCCGG TCTTGGTCAA GAAACCTACA TCCCCGAGGG TCTTCAGTGC    480
TTCCCACTTC AGCAAGGCAT GGGTGCTTCA CGTAAAGAGA CGGAAGAAGT AATCTTCGGA    540
GCTCTTGACA ATCTTTTTCG CAACACCGGT GTAAAACCTG ATGATATCGG TATATTGGTG    600
GTGAATTCTA GCACGTTTAA TCCAACTCCA TCACTCGCCT CCATGATTGT GAACAAGTAC    660
AAACTCAGAG ACAACATCAA GAGTTTGAAT CTTGGAGGGA TGGGTTGCAG TGCCGGAGTT    720
ATAGCTGTTG ATGTCGCTAA GGGATTACTA CAAGTTCATA GGAACACTTA TGCTATTGTA    780
GTAAGCACAG AGAACATCAC TCAGAACTTA TACTTGGGGA AAAACAAATC AATGCTAGTC    840
ACAAACTGTT TGTTCCGCGT TGGTGGTGCT GCGGTTCTGC TTTCAAACAG ATCTAGAGAC    900
CGTAACCGCG CCAAATACGA GCTTGTTCAC ACCGTACGGA TCCATACCGG ATCAGATGAT    960
AGGTCGTTCG AATGTGCGAC ACAAGAAGAG GATGAAGATG GTATAATTGG AGTTACCTTG   1020
ACAAAGAATC TACCTATGGT GGCTGCAAGG ACTCTTAAGA TAAATATCGC AACTTTGGGT   1080
CCTCTTGTAC TTCCATTAAA AGAGAAGCTA GCCTTCTTTA TTACTTTTGT CAAGAAGAAG   1140
TATTTCAAGC CAGAGTTAAG GAATTATACA CCAGATTTCA AGCTTGCCTT TGAGCATTTC   1200
TGTATCCACG CTGGTGGAAG AGCTCTAATA GATGAGCTGG AGAAGAACCT TAAGCTTTCT   1260
CCGTTACACG TAGAGGCGTC AAGAATGACA CTACACAGGT TTGGTAACAC TTCTTCTAGC   1320
TCAATCTGGT ACGAGTTAGC TTATACAGAA GCTAAAGGAA GGATGAAGGA AGGAGATAGG   1380
ATTTGGCAGA TTGCTTTGGG GTCAGGTTTT AAGTGTAACA GTTCAGTATG GGTGGCTCTG   1440
CGAGACGTTA AGCCTTCAGC TAACAGTCCA TGGGAAGACT GTATGGATAG ATATCCGGTT   1500
GAGATTGATA TT                                                       1512
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 504 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Leu Arg Gln Gly Arg Thr Lys Ser Lys His Leu Ser Lys Thr Ile Cys
 1               5                  10                  15
Pro Thr Leu Arg Leu Ser Pro Met Lys Asn Leu Lys Met Val Phe Phe
            20                  25                  30
```

```
Lys Ile Leu Phe Ile Ser Leu Met Ala Gly Leu Ala Met Lys Gly Ser
        35                  40                  45
Lys Ile Asn Val Glu Asp Leu Gln Lys Phe Ser Leu His His Thr Gln
    50                  55                  60
Asn Asn Leu Gln Thr Ile Ser Leu Leu Leu Phe Leu Val Val Phe Val
65                  70                  75                  80
Trp Ile Leu Tyr Met Leu Thr Arg Pro Lys Pro Val Tyr Leu Val Asp
                85                  90                  95
Phe Ser Cys Tyr Leu Pro Pro Ser His Leu Lys Val Ser Ile Gln Thr
            100                 105                 110
Leu Met Gly His Ala Arg Arg Ala Arg Glu Ala Gly Met Cys Trp Lys
        115                 120                 125
Asn Lys Glu Ser Asp His Leu Val Asp Phe Gln Glu Lys Ile Leu Glu
    130                 135                 140
Arg Ser Gly Leu Gly Gln Glu Thr Tyr Ile Pro Glu Gly Leu Gln Cys
145                 150                 155                 160
Phe Pro Leu Gln Gln Gly Met Gly Ala Ser Arg Lys Glu Thr Glu Glu
                165                 170                 175
Val Ile Phe Gly Ala Leu Asp Asn Leu Phe Arg Asn Thr Gly Val Lys
            180                 185                 190
Pro Asp Asp Ile Gly Ile Leu Val Val Asn Ser Ser Thr Phe Asn Pro
        195                 200                 205
Thr Pro Ser Leu Ala Ser Met Ile Val Asn Lys Tyr Lys Leu Arg Asp
    210                 215                 220
Asn Ile Lys Ser Leu Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val
225                 230                 235                 240
Ile Ala Val Asp Val Ala Lys Gly Leu Leu Gln Val His Arg Asn Thr
                245                 250                 255
Tyr Ala Ile Val Val Ser Thr Glu Asn Ile Thr Gln Asn Leu Tyr Leu
            260                 265                 270
Gly Lys Asn Lys Ser Met Leu Val Thr Asn Cys Leu Phe Arg Val Gly
        275                 280                 285
Gly Ala Ala Val Leu Leu Ser Asn Arg Ser Arg Asp Arg Asn Arg Ala
    290                 295                 300
Lys Tyr Glu Leu Val His Thr Val Arg Ile His Thr Gly Ser Asp Asp
305                 310                 315                 320
Arg Ser Phe Glu Cys Ala Thr Gln Glu Glu Asp Glu Asp Gly Ile Ile
                325                 330                 335
Gly Val Thr Leu Thr Lys Asn Leu Pro Met Val Ala Ala Arg Thr Leu
            340                 345                 350
Lys Ile Asn Ile Ala Thr Leu Gly Pro Leu Val Leu Pro Leu Lys Glu
        355                 360                 365
Lys Leu Ala Phe Phe Ile Thr Phe Val Lys Lys Lys Tyr Phe Lys Pro
    370                 375                 380
Glu Leu Arg Asn Tyr Thr Pro Asp Phe Lys Leu Ala Phe Glu His Phe
385                 390                 395                 400
Cys Ile His Ala Gly Gly Arg Ala Leu Ile Asp Glu Leu Glu Lys Asn
                405                 410                 415
Leu Lys Leu Ser Pro Leu His Val Glu Ala Ser Arg Met Thr Leu His
            420                 425                 430
Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala Tyr
        435                 440                 445
Thr Glu Ala Lys Gly Arg Met Lys Glu Gly Asp Arg Ile Trp Gln Ile
    450                 455                 460
Ala Leu Gly Ser Gly Phe Lys Cys Asn Ser Ser Val Trp Val Ala Leu
465                 470                 475                 480
Arg Asp Val Lys Pro Ser Ala Asn Ser Pro Trp Glu Asp Cys Met Asp
                485                 490                 495
Arg Tyr Pro Val Glu Ile Asp Ile
            500
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATGGGTAGAT CCAACGAGCA AGATCTGCTC TCTACCGAGA TCGTTAATCG TGGGATCGAA      60
CCATCCGGTC CTAACGCCGG CTCACCAACG TTCTCGGTTA GGGTCAGGAG ACGTTTGCCT     120
GATTTTCTTC AGTCGGTGAA CTTGAAGTAC GTGAAACTTG GTTACCACTA CCTCATAAAC     180
CATGCGGTTT ATTTGGCGAC CATACCGGTT CTTGTGCTGG TTTTTAGTGC TGAGGTTGGG     240
AGTTTAAGCA GAGAAGAGAT TTGGAAGAAG CTTTGGGACT ATGATCTTGC AACTGTTATC     300
GGATTCTTCG GTGTCTTTGT TTTAACCGCT TGTGTCTACT TCATGTCTCG TCCTCGCTCT     360
GTTTATCTTA TTGATTTCGC TTGTTACAAG CCCTCCGATG AACACAAGGT GACAAAAGAA     420
GAGTTCATAG AACTAGCGAG AAAATCAGGG AAGTTCGACG AAGAGACACT CGGTTTCAAG     480
AAGAGGATCT TACAAGCCTC AGGCATAGGC GACGAGACAT ACGTCCCAAG ATCCATCTCT     540
TCATCAGAAA ACATAACAAC GATGAAAGAA GGTCGTGAAG AAGCCTCTAC AGTGATCTTT     600
GGAGCACTAG ACGAACTCTT CGAGAAGACA CGTGTAAAAC CTAAAGACGT TGGTGTCCTT     660
GTGGTTAACT GTAGCATTTT CAACCCGACA CCGTCGTTGT CCGCAATGGT GATAAACCAT     720
TACAAGATGA GAGGGAACAT ACTTAGTTAC AACCTTGGAG GGATGGGATG TTCGGCTGGA     780
ATCATAGCTA TTGATCTTGC TCGTGACATG CTTCAGTCTA ACCCTAATAG TTATGCTGTT     840
GTTGTGAGTA CTGAGATGGT TGGGTATAAT TGGTACGTGG GAAGTGACAA GTCAATGGTT     900
ATACCTAATT GTTTCTTTAG GATGGGTTGT TCTGCCGTTA TGCTCTCTAA CCGTCGTCGT     960
GACTTTCGCC ATGCTAAGTA CCGTCTCGAG CACATTGTCC GAACTCATAA GGCTGCTGAC    1020
GACCGTAGCT TCAGGAGTGT GTACCAGGAA GAAGATGAAC AAGGATTCAA GGGGTTGAAG    1080
ATAAGTAGAG ACTTAATGGA AGTTGGAGGT GAAGCTCTCA AGACAAACAT CACTACCTTA    1140
GGTCCTCTTG TCCTACCTTT CTCCGAGCAG CTTCTCTTCT TTGCTGCTTT GGTCCGCCGA    1200
ACATTCTCAC CTGCTGCCAA AACGTCCACA ACCACTTCCT TCTCTACTTC CGCCACCGCA    1260
AAAACCAATG GAATCAAGTC TTCCTCTTCC GATCTGTCCA AGCCATACAT CCCGGACTAC    1320
AAGCTCGCCT TCGAGCATTT TTGCTTCCAC GCGGCAAGCA AAGTAGTGCT TGAAGAGCTT    1380
CAAAAGAATC TAGGCTTGAG TGAAGAGAAT ATGGAGGCTT CTAGGATGAC ACTTCACAGG    1440
TTTGGAAACA CTTCTAGCAG TGGAATCTGG TATGAGTTGG CTTACATGGA GGCCAAGGAA    1500
AGTGTTCGTA GAGGCGATAG GGTTTGGCAG ATCGCTTTCG GTTCTGGTTT TAAGTGTAAC    1560
AGTGTGGTGT GGAAGGCAAT GAGGAAGGTG AAGAAGCCAA CCAGGAACAA TCCTTGGGTG    1620
GATTGCATCA ACCGTTACCC TGTGCCTCTC                                     1650
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 550 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Gly Arg Ser Asn Glu Gln Asp Leu Leu Ser Thr Glu Ile Val Asn
 1               5                  10                  15
Arg Gly Ile Glu Pro Ser Gly Pro Asn Ala Gly Ser Pro Thr Phe Ser
            20                  25                  30
Val Arg Val Arg Arg Arg Leu Pro Asp Phe Leu Gln Ser Val Asn Leu
        35                  40                  45
Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu Ile Asn His Ala Val Tyr
    50                  55                  60
Leu Ala Thr Ile Pro Val Leu Val Leu Val Phe Ser Ala Glu Val Gly
65                  70                  75                  80
Ser Leu Ser Arg Glu Glu Ile Trp Lys Lys Leu Trp Asp Tyr Asp Leu
                85                  90                  95
Ala Thr Val Ile Gly Phe Phe Gly Val Phe Val Leu Thr Ala Cys Val
            100                 105                 110
```

```
Tyr Phe Met Ser Arg Pro Arg Ser Val Tyr Leu Ile Asp Phe Ala Cys
        115                 120                 125
Tyr Lys Pro Ser Asp Glu His Lys Val Thr Lys Glu Glu Phe Ile Glu
    130                 135                 140
Leu Ala Arg Lys Ser Gly Lys Phe Asp Glu Glu Thr Leu Gly Phe Lys
145                 150                 155                 160
Lys Arg Ile Leu Gln Ala Ser Gly Ile Gly Asp Glu Thr Tyr Val Pro
                165                 170                 175
Arg Ser Ile Ser Ser Ser Glu Asn Ile Thr Thr Met Lys Glu Gly Arg
            180                 185                 190
Glu Glu Ala Ser Thr Val Ile Phe Gly Ala Leu Asp Glu Leu Phe Glu
        195                 200                 205
Lys Thr Arg Val Lys Pro Lys Asp Val Gly Val Leu Val Val Asn Cys
    210                 215                 220
Ser Ile Phe Asn Pro Thr Pro Ser Leu Ser Ala Met Val Ile Asn His
225                 230                 235                 240
Tyr Lys Met Arg Gly Asn Ile Leu Ser Tyr Asn Leu Gly Gly Met Gly
                245                 250                 255
Cys Ser Ala Gly Ile Ile Ala Ile Asp Leu Ala Arg Asp Met Leu Gln
            260                 265                 270
Ser Asn Pro Asn Ser Tyr Ala Val Val Val Ser Thr Glu Met Val Gly
        275                 280                 285
Tyr Asn Trp Tyr Val Gly Ser Asp Lys Ser Met Val Ile Pro Asn Cys
    290                 295                 300
Phe Phe Arg Met Gly Cys Ser Ala Val Met Leu Ser Asn Arg Arg Arg
305                 310                 315                 320
Asp Phe Arg His Ala Lys Tyr Arg Leu Glu His Ile Val Arg Thr His
                325                 330                 335
Lys Ala Ala Asp Asp Arg Ser Phe Arg Ser Val Tyr Gln Glu Glu Asp
            340                 345                 350
Glu Gln Gly Phe Lys Gly Leu Lys Ile Ser Arg Asp Leu Met Glu Val
        355                 360                 365
Gly Gly Glu Ala Leu Lys Thr Asn Ile Thr Thr Leu Gly Pro Leu Val
    370                 375                 380
Leu Pro Phe Ser Glu Gln Leu Leu Phe Phe Ala Ala Leu Val Arg Arg
385                 390                 395                 400
Thr Phe Ser Pro Ala Ala Lys Thr Ser Thr Thr Thr Ser Phe Ser Thr
                405                 410                 415
Ser Ala Thr Ala Lys Thr Asn Gly Ile Lys Ser Ser Ser Ser Asp Leu
            420                 425                 430
Ser Lys Pro Tyr Ile Pro Asp Tyr Lys Leu Ala Phe Glu His Phe Cys
        435                 440                 445
Phe His Ala Ala Ser Lys Val Val Leu Glu Glu Leu Gln Lys Asn Leu
    450                 455                 460
Gly Leu Ser Glu Glu Asn Met Glu Ala Ser Arg Met Thr Leu His Arg
465                 470                 475                 480
Phe Gly Asn Thr Ser Ser Ser Gly Ile Trp Tyr Glu Leu Ala Tyr Met
                485                 490                 495
Glu Ala Lys Glu Ser Val Arg Arg Gly Asp Arg Val Trp Gln Ile Ala
            500                 505                 510
Phe Gly Ser Gly Phe Lys Cys Asn Ser Val Val Trp Lys Ala Met Arg
        515                 520                 525
Lys Val Lys Lys Pro Thr Arg Asn Asn Pro Trp Val Asp Cys Ile Asn
    530                 535                 540
Arg Tyr Pro Val Pro Leu
545                 550
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1611 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
TCGAGCTACG TCAGGGCTTT TATATGCACA AATTCTCATA AAGTTTTCAA TTTTATTCCA     60
TTTTTCTCGG AAGCCATGGA AGCTGCTAAT GAGCCTGTTA ATGGCGGATC CGTACAGATC    120
CGAACAGAGA ACAACGAAAG ACGAAAGCTT CCTAATTTCT TACAAAGCGT CAACATGAAA    180
TACGTCAAGC TAGGTTATCA TTACCTCATT ACTCATCTCT TCAAGCTCTG TTTGGTTCCA    240
TTAATGGCGG TTTTAGTCAC AGAGATCTCT CGATTAACAA CAGACGATCT TTACCAGATT    300
TGGCTTCATC TCCAATACAA TCTCGTTGCT TTCATCTTTC TCTCTGCTTT AGCTATCTTT    360
GGCTCCACCG TTTACATCAT GAGTCGTCCC AGATCTGTTT ATCTCGTTGA TTACTCTTGT    420
TATCTTCCTC CGGAGAGTCT TCAGGTTAAG TATCAGAAGT TTATGGATCA TTCTAAGTTG    480
ATTGAAGATT TCAATGAGTC ATCTTTAGAG TTTCAGAGGA AGATTCTTGA ACGTTCTGGT    540
TTAGGAGAAG AGACTTATCT CCCTGAAGCT TTACATTGTA TCCCTCCGAG GCCTACGATG    600
ATGGCGGCTC GTGAGGAATC TGAGCAGGTA ATGTTTGGTG CTCTTGATAA GCTTTTCGAG    660
AATACCAAGA TTAACCCTAG GGATATTGGT GTGTTGGTTG TGAATTGTAG CTTGTTTAAT    720
CCTACACCTT CGTTGTCAGC TATGATTGTT AACAAGTATA AGCTTAGAGG GAATGTTAAG    780
AGTTTTAACC TTGGTGGAAT GGGGTGTAGT GCTGGTGTTA TCTCTATCGA TTTAGCTAAA    840
GATATGTTGC AAGTTCATAG GAATACTTAT GCTGTTGTGG TTAGTACTGA GAACATTACT    900
CAGAATTGGT ATTTTGGGAA TAAGAAGGCT ATGTTGATTC CGAATTGTTT GTTTCGTGTT    960
GGTGGTTCGG CGATTTTGTT GTCGAACAAG GGGAAAGATC GTAGACGGTC TAAGTATAAG   1020
CTTGTTCATA CCGTTAGGAC TCATAAAGGA GCTGTTGAGA AGGCTTTCAA CTGTGTTTAC   1080
CAAGAGCAAG ATGATAATGG GAAGACCGGG GTTTCGTTGT CGAAAGATCT TATGGCTATA   1140
GCTGGGGAAG CTCTTAAGGC GAATATCACT ACTTTAGGTC CTTTGGTTCT TCCTATAAGT   1200
GAGCAGATTC TGTTTTTCAT GACTTTGGTT ACGAAGAAAC TGTTTAACTC GAAGCTGAAG   1260
CCGTATATTC CGGATTTCAA GCTTGCGTTT GATCATTTCT GTATCCATGC TGGTGGTAGA   1320
GCTGTGATTG ATGAGCTTGA GAAGAATCTG CAGCTTTCGC AGACTCATGT CGAGGCATCC   1380
AGAATGACAC TGCACAGATT TGGAAACACT TCTTCGAGCT CGATTTGGTA TGAACTGGCT   1440
TACATAGAGG CTAAAGGTAG GATGAAGAAA GGAAACCGGG TTTGGCAGAT TGCTTTTGGA   1500
AGTGGGTTTA AGTGTAACAG TGCAGTTTGG GTGGCTCTAA ACAATGTCAA GCCTTCGGTT   1560
AGTAGTCCGT GGGAACACTG CATCGACCGA TATCCGGTTA AGCTCGACTT C            1611
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 537 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Ser Ser Tyr Val Arg Ala Phe Ile Cys Thr Asn Ser His Lys Val Phe
 1               5                  10                  15
Asn Phe Ile Pro Phe Phe Ser Glu Ala Met Glu Ala Ala Asn Glu Pro
            20                  25                  30
Val Asn Gly Gly Ser Val Gln Ile Arg Thr Glu Asn Asn Glu Arg Arg
        35                  40                  45
Lys Leu Pro Asn Phe Leu Gln Ser Val Asn Met Lys Tyr Val Lys Leu
    50                  55                  60
Gly Tyr His Tyr Leu Ile Thr His Leu Phe Lys Leu Cys Leu Val Pro
65                  70                  75                  80
Leu Met Ala Val Leu Val Thr Glu Ile Ser Arg Leu Thr Thr Asp Asp
                85                  90                  95
Leu Tyr Gln Ile Trp Leu His Leu Gln Tyr Asn Leu Val Ala Phe Ile
            100                 105                 110
Phe Leu Ser Ala Leu Ala Ile Phe Gly Ser Thr Val Tyr Ile Met Ser
        115                 120                 125
Arg Pro Arg Ser Val Tyr Leu Val Asp Tyr Ser Cys Tyr Leu Pro Pro
    130                 135                 140
Glu Ser Leu Gln Val Lys Tyr Gln Lys Phe Met Asp His Ser Lys Leu
145                 150                 155                 160
Ile Glu Asp Phe Asn Glu Ser Ser Leu Glu Phe Gln Arg Lys Ile Leu
                165                 170                 175
Glu Arg Ser Gly Leu Gly Glu Glu Thr Tyr Leu Pro Glu Ala Leu His
            180                 185                 190
```

```
Cys Ile Pro Pro Arg Pro Thr Met Met Ala Ala Arg Glu Glu Ser Glu
        195                 200                 205
Gln Val Met Phe Gly Ala Leu Asp Lys Leu Phe Glu Asn Thr Lys Ile
    210                 215                 220
Asn Pro Arg Asp Ile Gly Val Leu Val Val Asn Cys Ser Leu Phe Asn
225                 230                 235                 240
Pro Thr Pro Ser Leu Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg
                245                 250                 255
Gly Asn Val Lys Ser Phe Asn Leu Gly Gly Met Gly Cys Ser Ala Gly
            260                 265                 270
Val Ile Ser Ile Asp Leu Ala Lys Asp Met Leu Gln Val His Arg Asn
        275                 280                 285
Thr Tyr Ala Val Val Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr
    290                 295                 300
Phe Gly Asn Lys Lys Ala Met Leu Ile Pro Asn Cys Leu Phe Arg Val
305                 310                 315                 320
Gly Gly Ser Ala Ile Leu Leu Ser Asn Lys Gly Lys Asp Arg Arg Arg
                325                 330                 335
Ser Lys Tyr Lys Leu Val His Thr Val Arg Thr His Lys Gly Ala Val
            340                 345                 350
Glu Lys Ala Phe Asn Cys Val Tyr Gln Glu Gln Asp Asp Asn Gly Lys
        355                 360                 365
Thr Gly Val Ser Leu Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Ala
    370                 375                 380
Leu Lys Ala Asn Ile Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser
385                 390                 395                 400
Glu Gln Ile Leu Phe Phe Met Thr Leu Val Thr Lys Lys Leu Phe Asn
                405                 410                 415
Ser Lys Leu Lys Pro Tyr Ile Pro Asp Phe Lys Leu Ala Phe Asp His
            420                 425                 430
Phe Cys Ile His Ala Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys
        435                 440                 445
Asn Leu Gln Leu Ser Gln Thr His Val Glu Ala Ser Arg Met Thr Leu
    450                 455                 460
His Arg Phe Gly Asn Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala
465                 470                 475                 480
Tyr Ile Glu Ala Lys Gly Arg Met Lys Lys Gly Asn Arg Val Trp Gln
                485                 490                 495
Ile Ala Phe Gly Ser Gly Phe Lys Cys Asn Ser Ala Val Trp Val Ala
            500                 505                 510
Leu Asn Asn Val Lys Pro Ser Val Ser Ser Pro Trp Glu His Cys Ile
        515                 520                 525
Asp Arg Tyr Pro Val Lys Leu Asp Phe
    530                 535
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1502 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TCTCCGACGA TGCCTCAGGC ACCGATGCCA GAGTTCTCTA GCTCGGTGAA GCTCAAGTAC     60
GTGAAACTTG GTTACCAATA TTTGGTTAAC CATTTCTTGA GTTTTCTTTT GATCCCGATC    120
ATGGCTATTG TCGCCGTTGA GCTTCTTCGG ATGGGTCCTG AAGAGATCCT TAATGTTTGG    180
AATTCACTCC AGTTTGACCT AGTTCAGGTT CTATGTTCTT CCTTCTTTGT CATCTTCATC    240
TCCACTGTTT ACTTCATGTC CAAGCCACGC ACCATCTACC TCGTTGACTA TTCTTGTTAC    300
AAGCCACCTG TCACGTGTCG TGTCCCCTTC GCAACTTTCA TGGAACACTC TCGTTTGATC    360
CTCAAGGACA AGCCTAAGAG CGTCGAGTTC CAAATGAGAA TCCTTGAACG TTCTGGCCTC    420
GGTGAGGAGA CTTGTCTCCC TCCGGCTATT CATTATATTC CTCCCACACC AACCATGGAC    480
GCGGCTAGAA GCGAGGCTCA GATGGTTATC TTCGAGGCCA TGGACGATCT TTTCAAGAAA    540
ACCGGTCTTA AACCTAAAGA CGTCGACATC CTTATCGTCA ACTGCTCTCT TTTCTCTCCC    600
```

```
ACACCATCGC TCTCAGCTAT GGTCATCAAC AAATATAAGC TTAGGAGTAA TATCAAGAGC    660
TTCAATCTTT CGGGGATGGG CTGCAGCGCG GGCCTGATCT CAGTTGATCT AGCCCGCGAC    720
TTGCTCCAAG TTCATCCCAA TTCAAATGCA ATCATCGTCA GCACGGAGAT CATAACGCCT    780
AATTACTATC AAGGCAACGA GAGAGCCATG TTGTTACCCA ATTGTCTCTT CCGCATGGGT    840
GCGGCAGCCA TACACATGTC AAACCGCCGG TCTGACCGGT GGCGAGCCAA ATACAAGCTT    900
TCCCACCTCG TCCGGACACA CCGTGGCGCT GACGACAAGT CTTTCTACTG TGTCTACGAA    960
CAGGAAGACA AAGAAGGACA CGTTGGCATC AACTTGTCCA AAGATCTCAT GGCCATCGCC   1020
GGTGAAGCCC TCAAGGCAAA CATCACCACA ATAGGTCCTT TGGTCCTACC GGCGTCAGAA   1080
CAACTTCTCT TCCTCACGTC CCTAATCGGA CGTAAAATCT TCAACCCGAA ATGGAAACCA   1140
TACATACCGG ATTTCAAGCT GGCCTTCGAA CACTTTTGCA TTCACGCAGG AGGCAGAGCG   1200
GTGATCGACG AGCTCCAAAA GAATCTACAA CTATCAGGAG AACACGTTGA GGCCTCAAGA   1260
ATGACACTAC ATCGTTTTGG TAACACGTCA TCTTCATCGT TATGGTACGA GCTTAGCTAC   1320
ATCGAGTCTA AAGGGAGAAT GAGGAGAGGC GATCGCGTTT GGCAAATCGC GTTTGGGAGT   1380
GGTTTCAAGT GTAACTCTGC CGTGTGGAAG TGTAACCGTA CGATTAAGAC ACCTAAGGAC   1440
GGACCATGGT CCGATTGTAT CGACCGTTAC CCTGTCTTTA TTCCCGAAGT TGTCAAACTC   1500
TA                                                                  1502
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Ser Pro Thr Met Pro Gln Ala Pro Met Pro Glu Phe Ser Ser Ser Val
 1               5                  10                  15
Lys Leu Lys Tyr Val Lys Leu Gly Tyr Gln Tyr Leu Val Asn His Phe
            20                  25                  30
Leu Ser Phe Leu Leu Ile Pro Ile Met Ala Ile Val Ala Val Glu Leu
        35                  40                  45
Leu Arg Met Gly Pro Glu Glu Ile Leu Asn Val Trp Asn Ser Leu Gln
    50                  55                  60
Phe Asp Leu Val Gln Val Leu Cys Ser Ser Phe Phe Val Ile Phe Ile
65                  70                  75                  80
Ser Thr Val Tyr Phe Met Ser Lys Pro Arg Thr Ile Tyr Leu Val Asp
                85                  90                  95
Tyr Ser Cys Tyr Lys Pro Pro Val Thr Cys Arg Val Pro Phe Ala Thr
            100                 105                 110
Phe Met Glu His Ser Arg Leu Ile Leu Lys Asp Lys Pro Lys Ser Val
        115                 120                 125
Glu Phe Gln Met Arg Ile Leu Glu Arg Ser Gly Leu Gly Glu Glu Thr
    130                 135                 140
Cys Leu Pro Pro Ala Ile His Tyr Ile Pro Pro Thr Pro Thr Met Asp
145                 150                 155                 160
Ala Ala Arg Ser Glu Ala Gln Met Val Ile Phe Glu Ala Met Asp Asp
                165                 170                 175
Leu Phe Lys Lys Thr Gly Leu Lys Pro Lys Asp Val Asp Ile Leu Ile
            180                 185                 190
Val Asn Cys Ser Leu Phe Ser Pro Thr Pro Ser Leu Ser Ala Met Val
        195                 200                 205
Ile Asn Lys Tyr Lys Leu Arg Ser Asn Ile Lys Ser Phe Asn Leu Ser
    210                 215                 220
Gly Met Gly Cys Ser Ala Gly Leu Ile Ser Val Asp Leu Ala Arg Asp
225                 230                 235                 240
Leu Leu Gln Val His Pro Asn Ser Asn Ala Ile Ile Val Ser Thr Glu
                245                 250                 255
Ile Ile Thr Pro Asn Tyr Tyr Gln Gly Asn Glu Arg Ala Met Leu Leu
            260                 265                 270
Pro Asn Cys Leu Phe Arg Met Gly Ala Ala Ala Ile His Met Ser Asn
        275                 280                 285
Arg Arg Ser Asp Arg Trp Arg Ala Lys Tyr Lys Leu Ser His Leu Val
    290                 295                 300
```

```
Arg Thr His Arg Gly Ala Asp Asp Lys Ser Phe Tyr Cys Val Tyr Glu
305                 310                 315                 320
Gln Glu Asp Lys Glu Gly His Val Gly Ile Asn Leu Ser Lys Asp Leu
                325                 330                 335
Met Ala Ile Ala Gly Glu Ala Leu Lys Ala Asn Ile Thr Thr Ile Gly
            340                 345                 350
Pro Leu Val Leu Pro Ala Ser Glu Gln Leu Leu Phe Leu Thr Ser Leu
        355                 360                 365
Ile Gly Arg Lys Ile Phe Asn Pro Lys Trp Lys Pro Tyr Ile Pro Asp
    370                 375                 380
Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala Gly Gly Arg Ala
385                 390                 395                 400
Val Ile Asp Glu Leu Gln Lys Asn Leu Gln Leu Ser Gly Glu His Val
                405                 410                 415
Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn Thr Ser Ser Ser
            420                 425                 430
Ser Leu Trp Tyr Glu Leu Ser Tyr Ile Glu Ser Lys Gly Arg Met Arg
        435                 440                 445
Arg Gly Asp Arg Val Trp Gln Ile Ala Phe Gly Ser Gly Phe Lys Cys
    450                 455                 460
Asn Ser Ala Val Trp Lys Cys Asn Arg Thr Ile Lys Thr Pro Lys Asp
465                 470                 475                 480
Gly Pro Trp Ser Asp Cys Ile Asp Arg Tyr Pro Val Phe Ile Pro Glu
                485                 490                 495
Val Val Lys Leu
            500
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1548 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
ATGGACGGTG CCGGAGAATC ACGACTCGGT GGTGATGGTG GTGGTGATGG TTCTGTTGGA      60
GTTCAGATCC GACAAACACG GATGCTACCG GATTTTCTCC AGAGCGTGAA TCTCAAGTAT     120
GTGAAATTAG GTTACCATTA CTTAATCTCA AATCTCTTGA CTCTCTGTTT ATTCCCTCTC     180
GCCGTTGTTA TCTCCGTCGA AGCCTCTCAG ATGAACCCAG ATGATCTCAA ACAGCTCTGG     240
ATCCATCTAC AATACAATCT GGTTAGTATC ATCATCTGTT CAGCGATTCT AGTCTTCGGG     300
TTAACGGTTT ATGTTATGAC CCGACCTAGA CCCGTTTACT TGGTTGATTT CTCTTGTTAT     360
CTCCCACCTG ATCATCTCAA AGCTCCTTAC GCTCGGTTCA TGGAACATTC TAGACTCACC     420
GGAGATTTCG ATGACTCTGC TCTCGAGTTT CAACGCAAGA TCCTTGAGCG TTCTGGTTTA     480
GGGGAAGACA CTTATGTCCC TGAAGCTATG CATTATGTTC CACCGAGAAT TTCAATGGCT     540
GCTGCTAGAG AAGAAGCTGA ACAAGTCATG TTTGGTGCTT TAGATAACCT TTTCGCTAAC     600
ACTAATGTGA AACCAAAGGA TATTGGAATC CTTGTTGTGA ATTGTAGTCT CTTTAATCCA     660
ACTCCTTCGT TATCTGCAAT GATTGTGAAC AAGTATAAGC TTAGAGGTAA CATTAGAAGC     720
TACAATCTAG GCGGTATGGG TTGCAGCGCG GGAGTTATCG CTGTGGATCT TGCTAAAGAC     780
ATGTTGTTGG TACATAGGAA CACTTATGCG GTTGTTGTTT CTACTGAGAA CATTACTCAG     840
AATTGGTATT TTGGTAACAA GAAATCGATG TTGATACCGA ACTGCTTGTT TCGAGTTGGT     900
GGCTCTGCGG TTTTGCTATC GAACAAGTCG AGGGACAAGA GACGGTCTAA GTACAGGCTT     960
GTACATGTAG TCAGGACTCA CCGTGGAGCA GATGATAAAG CTTTCCGTTG TGTTTATCAA    1020
GAGCAGGATG ATACAGGGAG AACCGGGGTT TCGTTGTCGA AAGATCTAAT GGCGATTGCA    1080
GGGGAAACTC TCAAAACCAA TATCACTACA TTGGGTCCTC TTGTTCTACC GATAAGTGAG    1140
CAGATTCTCT TCTTTATGAC TCTAGTTGTG AAGAAGCTCT TTAACGGTAA AGTGAAACCG    1200
TATATCCCGG ATTTCAAACT TGCTTTCGAG CATTTCTGTA TCCATGCTGG TGGAAGAGCT    1260
GTGATCGATG AGTTAGAGAA GAATCTGCAG CTTTCACCAG TTCATGTCGA GGCTTCGAGG    1320
ATGACTCTTC ATCGATTTGG TAACACATCT TCGAGCTCCA TTTGGTATGA ATTGGCTTAC    1380
ATTGAAGCGA AGGGAAGGAT GCGAAGAGGT AATCGTGTTT GGCAAATCGC GTTCGGAAGT    1440
GGATTTAAAT GTAATAGCGC GATTTGGGAA GCATTAAGGC ATGTGAAACC TTCGAACAAC    1500
AGTCCTTGGG AAGATTGTAT TGACAAGTAT CCGGTAACTT TAAGTTAT                 1548
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 516 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Asp Gly Ala Gly Glu Ser Arg Leu Gly Gly Asp Gly Gly Gly Asp
 1               5                  10                  15
Gly Ser Val Gly Val Gln Ile Arg Gln Thr Arg Met Leu Pro Asp Phe
            20                  25                  30
Leu Gln Ser Val Asn Leu Lys Tyr Val Lys Leu Gly Tyr His Tyr Leu
        35                  40                  45
Ile Ser Asn Leu Leu Thr Leu Cys Leu Phe Pro Leu Ala Val Val Ile
    50                  55                  60
Ser Val Glu Ala Ser Gln Met Asn Pro Asp Asp Leu Lys Gln Leu Trp
65                  70                  75                  80
Ile His Leu Gln Tyr Asn Leu Val Ser Ile Ile Ile Cys Ser Ala Ile
                85                  90                  95
Leu Val Phe Gly Leu Thr Val Tyr Val Met Thr Arg Pro Arg Pro Val
            100                 105                 110
Tyr Leu Val Asp Phe Ser Cys Tyr Leu Pro Pro Asp His Leu Lys Ala
        115                 120                 125
Pro Tyr Ala Arg Phe Met Glu His Ser Arg Leu Thr Gly Asp Phe Asp
    130                 135                 140
Asp Ser Ala Leu Glu Phe Gln Arg Lys Ile Leu Glu Arg Ser Gly Leu
145                 150                 155                 160
Gly Glu Asp Thr Tyr Val Pro Glu Ala Met His Tyr Val Pro Pro Arg
                165                 170                 175
Ile Ser Met Ala Ala Ala Arg Glu Glu Ala Glu Gln Val Met Phe Gly
            180                 185                 190
Ala Leu Asp Asn Leu Phe Ala Asn Thr Asn Val Lys Pro Lys Asp Ile
        195                 200                 205
Gly Ile Leu Val Val Asn Cys Ser Leu Phe Asn Pro Thr Pro Ser Leu
    210                 215                 220
Ser Ala Met Ile Val Asn Lys Tyr Lys Leu Arg Gly Asn Ile Arg Ser
225                 230                 235                 240
Tyr Asn Leu Gly Gly Met Gly Cys Ser Ala Gly Val Ile Ala Val Asp
                245                 250                 255
Leu Ala Lys Asp Met Leu Leu Val His Arg Asn Thr Tyr Ala Val Val
            260                 265                 270
Val Ser Thr Glu Asn Ile Thr Gln Asn Trp Tyr Phe Gly Asn Lys Lys
        275                 280                 285
Ser Met Leu Ile Pro Asn Cys Leu Phe Arg Val Gly Gly Ser Ala Val
    290                 295                 300
Leu Leu Ser Asn Lys Ser Arg Asp Lys Arg Arg Ser Lys Tyr Arg Leu
305                 310                 315                 320
Val His Val Val Arg Thr His Arg Gly Ala Asp Asp Lys Ala Phe Arg
                325                 330                 335
Cys Val Tyr Gln Glu Gln Asp Asp Thr Gly Arg Thr Gly Val Ser Leu
            340                 345                 350
Ser Lys Asp Leu Met Ala Ile Ala Gly Glu Thr Leu Lys Thr Asn Ile
        355                 360                 365
Thr Thr Leu Gly Pro Leu Val Leu Pro Ile Ser Glu Gln Ile Leu Phe
    370                 375                 380
Phe Met Thr Leu Val Val Lys Lys Leu Phe Asn Gly Lys Val Lys Pro
385                 390                 395                 400
Tyr Ile Pro Asp Phe Lys Leu Ala Phe Glu His Phe Cys Ile His Ala
                405                 410                 415
Gly Gly Arg Ala Val Ile Asp Glu Leu Glu Lys Asn Leu Gln Leu Ser
            420                 425                 430
Pro Val His Val Glu Ala Ser Arg Met Thr Leu His Arg Phe Gly Asn
        435                 440                 445
```

```
Thr Ser Ser Ser Ser Ile Trp Tyr Glu Leu Ala Tyr Ile Glu Ala Lys
    450                 455                 460
Gly Arg Met Arg Arg Gly Asn Arg Val Trp Gln Ile Ala Phe Gly Ser
465                 470                 475                 480
Gly Phe Lys Cys Asn Ser Ala Ile Trp Glu Ala Leu Arg His Val Lys
                485                 490                 495
Pro Ser Asn Asn Ser Pro Trp Glu Asp Cys Ile Asp Lys Tyr Pro Val
            500                 505                 510
Thr Leu Ser Tyr
        515
```

WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.

2. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:2.

3. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:4.

4. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:6.

5. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:8.

6. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:10.

7. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:12.

8. The polynucleotide of claim 1, wherein said amino acid sequence is SEQ ID NO:14.

9. An isolated polynucleotide, wherein said polynucleotide is selected from the group consisting of:

a) SEQ ID NO:1;

b) SEQ ID NO:3;

c) SEQ ID NO:5;

d) SEQ ID NO:7;

e) SEQ ID NO:9;

f) SEQ ID NO:11;

g) SEQ ID NO:13;

h) an RNA analog of SEQ ID NO:1;

i) an RNA analog of SEQ ID NO:3;

j) an RNA analog of SEQ ID NO:5;

k) an RNA analog of SEQ ID NO:7;

l) an RNA analog of SEQ ID NO:9;

m) an RNA analog of SEQ ID NO:11;

n) an RNA analog of SEQ ID NO:13;

o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and

p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

10. An isolated polypeptide having an amino acid sequence selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an

amino acid sequence substantially identical to SEQ ID NO:14.

11. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:2.

12. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:4.

13. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:6.

14. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:8.

15. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:10.

16. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:12.

17. The polypeptide of claim 10, wherein said amino acid sequence is SEQ ID NO:14.

18. A transgenic plant containing a nucleic acid construct comprising a polynucleotide selected from the group consisting of:

a) SEQ ID NO:1;
b) SEQ ID NO:3;
c) SEQ ID NO:5;
d) SEQ ID NO:7;
e) SEQ ID NO:9;
f) SEQ ID NO:11;
g) SEQ ID NO:13;
h) an RNA analog of SEQ ID NO:1;

i) an RNA analog of SEQ ID NO:3;

j) an RNA analog of SEQ ID NO:5;

k) an RNA analog of SEQ ID NO:7;

l) an RNA analog of SEQ ID NO:9;

m) an RNA analog of SEQ ID NO:11;

n) an RNA analog of SEQ ID NO:13;

o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and

p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

19. The plant of claim 18, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.

20. The plant of claim 19, wherein said regulatory element is a tissue-specific promoter.

21. The plant of claim 20, wherein said regulatory element is an epidermal cell-specific promoter.

22. The plant of claim 20, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.

23. The plant of claim 22, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.

24. A transgenic plant containing a nucleic acid construct comprising a polynucleotide encoding a polypeptide selected from the group consisting of: an amino acid sequence substantially identical to SEQ ID NO:2, an amino acid sequence substantially identical to SEQ ID NO:4, an amino acid sequence substantially identical to SEQ ID NO:6, an amino acid sequence substantially identical to SEQ ID NO:8, an amino acid sequence substantially identical to SEQ ID NO:10, an amino acid sequence substantially identical to SEQ ID NO:12, and an amino acid sequence substantially identical to SEQ ID NO:14.

25. The plant of claim 24, wherein said construct further comprises a regulatory element operably linked to said polynucleotide.

26. The plant of claim 25, wherein said regulatory element is a tissue-specific promoter.

27. The plant of claim 26, wherein said regulatory element is an epidermal cell-specific promoter.

28. The plant of claim 26, wherein said regulatory element is a seed-specific promoter that is operably linked in sense orientation to said polynucleotide.

29. The plant of claim 28, wherein said plant has altered levels of very long chain fatty acids in seeds compared to the levels in a plant lacking said nucleic acid construct.

30. A method of altering the levels of very long chain fatty acids in a plant, comprising the steps of:

A) creating a nucleic acid construct, said construct comprising a polynucleotide selected from the group consisting of:

a) SEQ ID NO:1;

b) SEQ ID NO:3;

c) SEQ ID NO:5;

d) SEQ ID NO:7;

e) SEQ ID NO:9;

f) SEQ ID NO:11;

g) SEQ ID NO:13;

h) an RNA analog of SEQ ID NO:1;

i) an RNA analog of SEQ ID NO:3;

j) an RNA analog of SEQ ID NO:5;

k) an RNA analog of SEQ ID NO:7;

l) an RNA analog of SEQ ID NO:9;

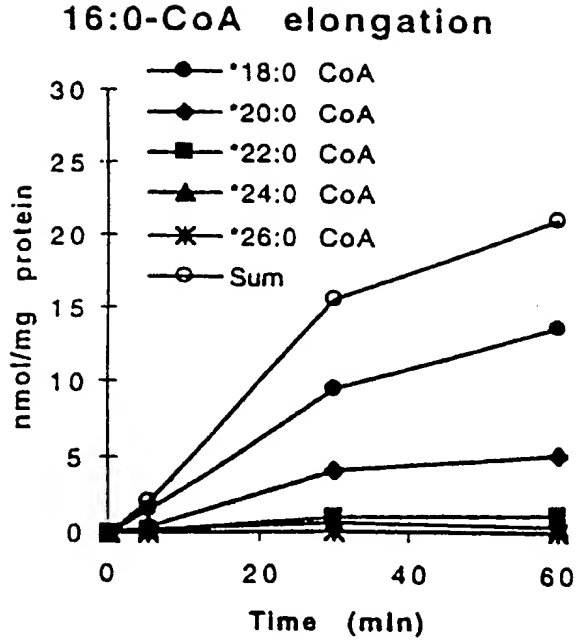
m) an RNA analog of SEQ ID NO:11;

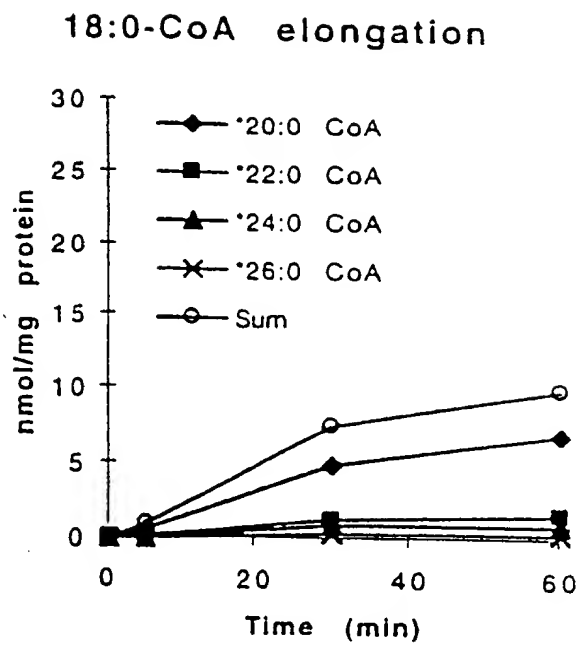
n) an RNA analog of SEQ ID NO:13;

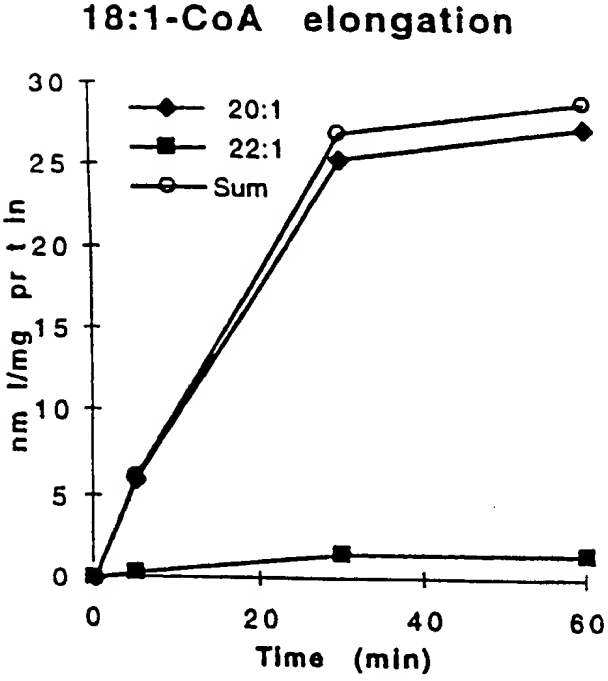
o) a polynucleotide having a nucleic acid sequence complementary to a), b), c), d), e), f), g), h), i), j), k), l), m), or n); and

p) a nucleic acid fragment of a), b), c), d), e), f), g), h), i), j), k), l), m), n), or o) that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14; and

B) introducing said construct into said plant, wherein said polynucleotide is effective for altering the levels of very long chain fatty acids in said plant.



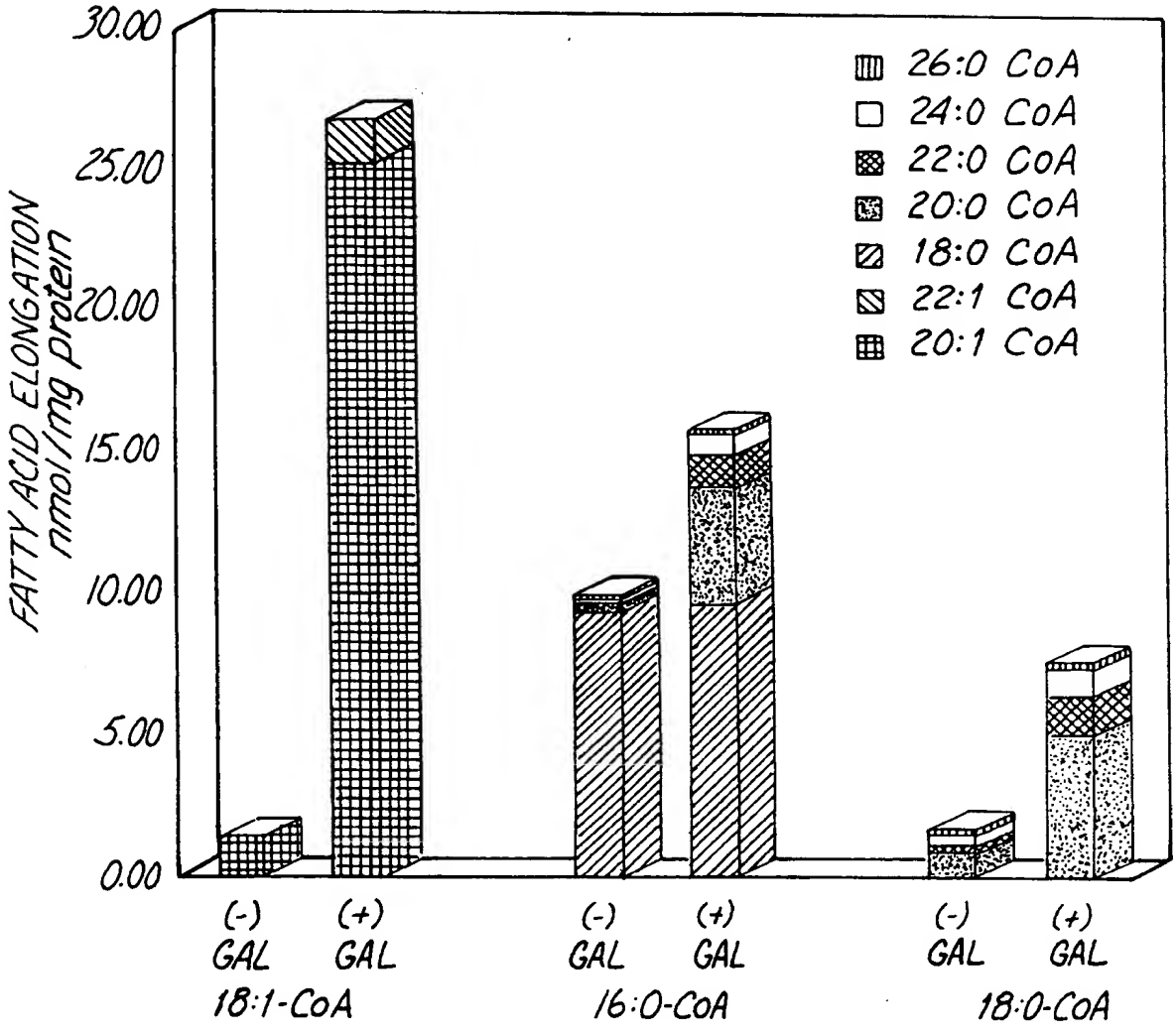




FAE1 W/RESPECT TO TIME

FIGURE 1

FIGURE 2


30.00
25.00
20.00
15.00
10.00
5.00
0.00
FATTY ACID ELONGATION
nmol/mg protein
26:0 CoA
24:0 CoA
22:0 CoA
20:0 CoA
18:0 CoA
22:1 CoA
20:1 CoA
(-) GAL
(+) GAL
18:1-CoA
16:0-CoA
18:0-CoA

EL1 1560 bases

ATGGATCGAG AGAGATTAAC GGCGGAGATG GCGTTTCGAG ATTCATCATC GGCCGTTATA
AGAATTCGAA GACGTTTGCC GGATTTATTA ACGTCCGTTA AGCTCAAATA CGTGAAGCTT
GGACTTCACA ACTCTTGCAA CGTGACCACC ATTCTCTTCT TCTTAATTAT TCTTCCTTTA
ACCGGAACCG TGCTGGTTCA GCTAACCGGT CTAACGTTCG ATACGTTCTC TGAGCTTTGG
TCTAACCAGG CGGTTCAACT CGACACGGCG ACGAGACTTA CCTGCTTGGT TTTCCTCTCC
TTCGTTTTGA CCCTCTACGT GGCTAACCGG TCTAAACCGG TTTACCTAGT GGATTTCTCC
TGCTACAAAC CGGAAGACGA GCGTAAAATA TCAGTAGATT CGTTCTTGAC GATGACTGAG
GAAAATGGAT CATTCACCGA TGACACGGTT CAGTTCCAGC AAAGAATCTC GAACCGGGCC
GGTTTGGGAG ACGAGACGTA TCTGCCACGT GGCATAACTT CAACGCCCCC GAAGCTAAAT
ATGTCAGAGG CACGTGCCGA AGCTGAAGCC GTTATGTTTG GAGCCTTAGA TTCCCTCTTC
GAGAAAACCG GAATTAAACC GGCCGAAGTC GGAATCTTGA TAGTAAACTG CAGCTTATTC
AATCCGACGC CGTCTCTATC AGCGATGATC GTGAACCATT ACAAGATGAG AGAAGACATC
AAAAGTTACA ACCTCGGAGG AATGGGTTGC TCCGCCGGAT TAATCTCAAT CGATCTCGCT
AACAATCTCC TCAAAGCAAA CCCTAATTCT TACGCTGTCG TGGTAAGCAC GGAAAACATA
ACCCTAAACT GGTACTTCGG AAATGACCGG TCAATGCTCC TCTGCAACTG CATCTTCCGA
ATGGGCGGAG CTGCGATTCT CCTCTCTAAC CGCCGTCAAG ACCGGAAGAA GTCAAAGTAC
TCGCTGGTCA ACGTCGTTCG AACACATAAA GGATCAGACG ACAAGAACTA CAATTGCGTG
TACCAGAAGG AAGACGAGAG AGGAACAATC GGTGTCTCTT TAGCTAGAGA GCTCATGTCT
GTCGCCGGAG ACGCTCTGAA AACAAACATC ACGACTTTAG GACCGATGGT TCTTCCATTG
TCAGAGCAGT TGATGTTCTT GATTTCCTTG GTCAAAAGGA AGATGTTCAA GTTAAAAGTT
AAACCGTATA TTCCGGATTT CAAGCTAGCT TTCGAGCATT TCTGTATTCA CGCAGGAGGT
AGAGCGGTTC TAGACGAAGT GCAGAAGAAT CTTGATCTCA AAGATTGGCA CATGGAACCT
TCTAGAATGA CTTTGCACAG ATTTGGTAAC ACTTCGAGTA GCTCGCTTTG GTATGAGATG
GCTTATACCG AAGCTAAGGG TCGGGTTAAA GCTGGTGACC GACTTTGGCA GATTGCGTTT
GGATCGGGTT TCAAGTGTAA TAGTGCGGTT TGGAAAGCGT TACGACCGGT TTCGACGGAG
GAGATGACCG GTAATGCTTG GGCTGGTTCG ATTGATCAAT ATCCGGTTAA AGTTGTGCAA

EL1

FIGURE 3

```
EL1 sequence
Molecular Weight 58379.00 Daltons
520 Amino Acids
62 Strongly Basic(+) Amino Acids (K,R)
52 Strongly Acidic(-) Amino Acids (D,E)
187 Hydrophobic Amino Acids (A,I,L,F,W,V)
144 Polar Amino Acids (N,C,Q,S,T,Y)
8.784 Isolectric Point
10.804 Charge at PH 7.0

MDRERLTAEM AFRDSSSAVI RIRRRLPDLL TSVKLKYVKL GLHNSCNVTT ILFFLIILPL
TGTVLVQLTG LTFDTFSELW SNQAVQLDTA TRLTCLVFLS FVLTLYVANR SKPVYLVDFS
CYKPEDERKI SVDSFLTMTE ENGSFTDDTV QFQQRISNRA GLGDETYLPR GITSTPPKLN
MSEARAEAEA VMFGALDSLF EKTGIKPAEV GILIVNCSLF NPTPSLSAMI VNHYKMREDI
KSYNLGGMGC SAGLISIDLA NNLLKANPNS YAVVVSTENI TLNWYFGNDR SMLLCNCIFR
MGGAAILLSN RRQDRKKSKY SLVNVVRTHK GSDDKNYNCV YQKEDERGTI GVSLARELMS
VAGDALKTNI TTLGPMVLPL SEQLMFLISL VKRKMFKLKV KPYIPDFKLA FEHFCIHAGG
RAVLDEVQKN LDLKDWHMEP SRMTLHRFGN TSSSSLWYEM AYTEAKGRVK AGDRLWQIAF
GSGFKCNSAV WKALRPVSTE EMTGNAWAGS IDQYPVKVVQ
```

FIGURE 4

EL2 1479 bases

```
ATGGATTACC CCATGAAGAA GGTAAAAATC TTTTTCAACT ACCTCATGGC GCATCGCTTC
AAGCTCTGCT TCTTACCATT AATGGTTGCT ATAGCCGTGG AGGCGTCTCG TCTTTCCACA  120
CAAGATCTCC AAAACTTTTA CCTCTACTTA CAAAACAACC ACACATCTCT AACCATGTTC
TTCCTTTACC TCGCTCTCGG GTCGACTCTT TACCTCATGA CCCGGCCCAA ACCCGTTTAT  240
CTCGTTGACT TTAGCTGCTA CCTCCCACCG TCGCATCTCA AAGCCAGCAC CCAGAGGATC
ATGCAACACG TAAGGCTTGT ACGAGAAGCA GGCGCGTGGA AGCAAGAGTC CGATTACTTG  360
ATGGACTTCT GCGAGAAGAT TCTAGAACGT TCCGGTCTAG GCCAAGAGAC GTACGTACCC
GAAGGTCTTC AAACTTTGCC ACTACAACAG AATTTGGCTG TATCACGTAT AGAGACGGAG  480
GAAGTTATTA TTGGTGCGGT CGATAATCTG TTTCGCAACA CGGGAATAAG CCCTAGTGAT
ATAGGTATAT TGGTGGTGAA TTCAAGCACT TTTAATCCAA CACCTTCGCT ATCAAGTATC  600
TTAGTGAATA AGTTTAAACT TAGGGATAAT ATAAAGAGCT TGAATCTTGG TGGGATGGGG
TGTAGCGCTG GAGTCATCGC TATCGATGCG GCTAAGAGCT TGTTACAAGT TCATAGAAAC  720
ACTTATGCTC TTGTGGTGAG CACGGAGAAC ATCACTCAAA ACTTGTACAT GGGTAACAAC
AAATCAATGT TGGTTACAAA CTGTTTGTTC CGTATAGGTG GGGCCGCGAT TTTGCTTTCT  840
AACCGGTCTA TAGATCGTAA ACGCGCAAAA TACGAGCTTG TTCACACCGT GCGGGTCCAT
ACCGGAGCAG ATGACCGATC CTATGAATGT GCAACTCAAG AAGAGGATGA AGATGGCATA  960
GTTGGGGTTT CCTTGTCAAA GAATCTACCA ATGGTAGCTG CAAGAACCCT AAAGATCAAT
ATCGCAACTT TGGGTCCGCT TGTTCTTCCC ATAAGCGAGA AGTTTCACTT CTTTGTGAGG 1080
TTCGTTAAAA AGAAGTTTCT CAACCCCAAG CTAAAGCATT ACATTCCGGA TTTCAAGCTC
GCATTCGAGC ATTTCTGTAT CCATGCGGGT GGTAGAGCGC TAATTGATGA GATGGAGAAG 1200
AATCTTCATC TAACTCCACT AGACGTTGAG GCTTCAAGAA TGACATTACA CAGGTTTGGT
AATACCTCTT CGAGCTCCAT TTGGTACGAG TTGGCTTACA CAGAAGCCAA AGGAAGGATG 1320
ACGAAAGGAG ATAGGATTTG GCAGATTGCG TTGGGGTCAG GTTTTAAGTG TAATAGTTCA
GTTTGGGTGG CTCTTCGTAA CGTCAAGCCT TCTACTAATA ATCCTTGGGA ACAGTGTCTA 1440
CACAAATATC CAGTTGAGAT CGATATAGAT TTAAAAGAG
```

EL2
FIGURE 5

EL2 protein sequence
Molecular Weight 55799.30 Daltons
493 Amino Acids
55 Strongly Basic(+) Amino Acids (K,R)
46 Strongly Acidic(-) Amino Acids (D,E)
181 Hydrophobic Amino Acids (A,I,L,F,W,V)
134 Polar Amino Acids (N,C,Q,S,T,Y)
8.756 Isolectric Point
10.995 Charge at PH 7.0

MDYPMKKVKI FFNYLMAHRF KLCFLPLMVA IAVEASRLST QDLQNFYLYL QNNHTSLTMF FLYLALGSTL
YLMTRPKPVY LVDFSCYLPP SHLKASTQRI MQHVRLVREA GAWKQESDYL MDFCEKILER SGLGQETYVP
EGLQTLPLQQ NLAVSRIETE EVIIGAVDNL FRNTGISPSD IGILVVNSST FNPTPSLSSI LVNKFKLRDN
IKSLNLGGMG CSAGVIAIDA AKSLLQVHRN TYALVVSTEN ITQNLYMGNN KSMLVTNCLF RIGGAAILLS
NRSIDRKRAK YELVHTVRVH TGADDRSYEC ATQEEDEDGI VGVSLSKNLP MVAARTLKIN IATLGPLVLP
ISEKFHFFVR FVKKKFLNPK LKHYIPDFKL AFEHFCIHAG GRALIDEMEK NLHLTPLDVE ASRMTLHRFG
NTSSSSIWYE LAYTEAKGRM TKGDRIWQIA LGSGFKCNSS VWVALRNVKP STNNPWEQCL HKYPVEIDID
LKE

FIGURE 6

EL3 1512 bases

CTACGTCAGG GTAGAACAAA GAGTAAACAC TTAAGCAAAA CAATTTGTCC TACTCTTAGG TTATCTCCAA
TGAAGAACTT AAAGATGGTT TTCTTCAAGA TCCTCTTTAT CTCTTTAATG GCAGGATTAG CCATGAAAGG
ATCTAAGATC AACGTAGAAG ATCTCCAAAA GTTCTCCCTC CACCATACAC AGAACAACCT CCAAACCATA
AGCCTTCTAT TGTTTCTTGT CGTTTTTGTG TGGATCCTCT ACATGTTAAC CCGACCTAAA CCCGTTTACC
TTGTTGATTT CTCCTGCTAC CTTCCACCGT CGCATCTCAA GGTCAGTATC CAAACCCTAA TGGGACACGC
AAGACGTGCA AGAGAAGCAG GCATGTGTTG GAAGAACAAA GAGAGCGACC ATTTAGTTGA CTTCCAGGAG
AAGATTCTTG AACGTTCCGG TCTTGGTCAA GAAACCTACA TCCCCGAGGG TCTTCAGTGC TTCCCACTTC
AGCAAGGCAT GGGTGCTTCA CGTAAAGAGA CGGAAGAAGT AATCTTCGGA GCTCTTGACA ATCTTTTTCG
CAACACCGGT GTAAAACCTG ATGATATCGG TATATTGGTG GTGAATTCTA GCACGTTTAA TCCAACTCCA
TCACTCGCCT CCATGATTGT GAACAAGTAC AAACTCAGAG ACAACATCAA GAGTTTGAAT CTTGGAGGGA
TGGGTTGCAG TGCCGGAGTT ATAGCTGTTG ATGTCGCTAA GGGATTACTA CAAGTTCATA GGAACACTTA
TGCTATTGTA GTAAGCACAG AGAACATCAC TCAGAACTTA TACTTGGGGA AAAACAAATC AATGCTAGTC
ACAAACTGTT TGTTCCGCGT TGGTGGTGCT GCGGTTCTGC TTTCAAACAG ATCTAGAGAC CGTAACCGCG
CCAAATACGA GCTTGTTCAC ACCGTACGGA TCCATACCGG ATCAGATGAT AGGTCGTTCG AATGTGCGAC
ACAAGAAGAG GATGAAGATG GTATAATTGG AGTTACCTTG ACAAAGAATC TACCTATGGT GGCTGCAAGG
ACTCTTAAGA TAAATATCGC AACTTTGGGT CCTCTTGTAC TTCCATTAAA AGAGAAGCTA GCCTTCTTTA
TTACTTTTGT CAAGAAGAAG TATTTCAAGC CAGAGTTAAG GAATTATACA CCAGATTTCA AGCTTGCCTT
TGAGCATTTC TGTATCCACG CTGGTGGAAG AGCTCTAATA GATGAGCTGG AGAAGAACCT TAAGCTTTCT
CCGTTACACG TAGAGGCGTC AAGAATGACA CTACACAGGT TTGGTAACAC TTCTTCTAGC TCAATCTGGT
ACGAGTTAGC TTATACAGAA GCTAAAGGAA GGATGAAGGA AGGAGATAGG ATTTGGCAGA TTGCTTTGGG
GTCAGGTTTT AAGTGTAACA GTTCAGTATG GGTGGCTCTG CGAGACGTTA AGCCTTCAGC TAACAGTCCA
TGGGAAGACT GTATGGATAG ATATCCGGTT GAGATTGATA TT

EL3

FIGURE 7

EL3 protein sequence
Molecular Weight 56801.10 Daltons
504 Amino Acids
66 Strongly Basic(+) Amino Acids (K,R)
48 Strongly Acidic(-) Amino Acids (D,E)
183 Hydrophobic Amino Acids (A,I,L,F,W,V)
127 Polar Amino Acids (N,C,Q,S,T,Y)
9.315 Isolectric Point
19.797 Charge at PH 7.0

LRQGRTKSKH LSKTICPTLR LSPMKNLKMV FFKILFISLM AGLAMKGSKI NVEDLQKFSL HHTQNNLQTI
SLLLFLVVFV WILYMLTRPK PVYLVDFSCY LPPSHLKVSI QTLMGHARRA REAGMCWKNK ESDHLVDFQE
KILERSGLGQ ETYIPEGLQC FPLQQGMGAS RKETEEVIFG ALDNLFRNTG VKPDDIGILV VNSSTFNPTP
SLASMIVNKY KLRDNIKSLN LGGMGCSAGV IAVDVAKGLL QVHRNTYAIV VSTENITQNL YLGKNKSMLV
TNCLFRVGGA AVLLSNRSRD RNRAKYELVH TVRIHTGSDD RSFECATQEE DEDGIIGVTL TKNLPMVAAR
TLKINIATLG PLVLPLKEKL AFFITFVKKK YFKPELRNYT PDFKLAFEHF CIHAGGRALI DELEKNLKLS
PLHVEASRMT LHRFGNTSSS SIWYELAYTE AKGRMKEGDR IWQIALGSGF KCNSSVWVAL RDVKPSANSP
WEDCMDRYPV EIDI

EL3
FIGURE 8

EL4 cDNA 1650 bases

ATGGGTAGAT CCAACGAGCA AGATCTGCTC TCTACCGAGA TCGTTAATCG TGGGATCGAA CCATCCGGTC
CTAACGCCGG CTCACCAACG TTCTCGGTTA GGGTCAGGAG ACGTTTGCCT GATTTTCTTC AGTCGGTGAA
CTTGAAGTAC GTGAAACTTG GTTACCACTA CCTCATAAAC CATGCGGTTT ATTTGGCGAC CATACCGGTT
CTTGTGCTGG TTTTTAGTGC TGAGGTTGGG AGTTTAAGCA GAGAAGAGAT TTGGAAGAAG CTTTGGGACT
ATGATCTTGC AACTGTTATC GGATTCTTCG GTGTCTTTGT TTTAACCGCT TGTGTCTACT TCATGTCTCG
TCCTCGCTCT GTTTATCTTA TTGATTTCGC TTGTTACAAG CCCTCCGATG AACACAAGGT GACAAAAGAA
GAGTTCATAG AACTAGCGAG AAAATCAGGG AAGTTCGACG AAGAGACACT CGGTTTCAAG AAGAGGATCT
TACAAGCCTC AGGCATAGGC GACGAGACAT ACGTCCCAAG ATCCATCTCT TCATCAGAAA ACATAACAAC
GATGAAAGAA GGTCGTGAAG AAGCCTCTAC AGTGATCTTT GGAGCACTAG ACGAACTCTT CGAGAAGACA
CGTGTAAAAC CTAAAGACGT TGGTGTCCTT GTGGTTAACT GTAGCATTTT CAACCCGACA CCGTCGTTGT
CCGCAATGGT GATAAACCAT TACAAGATGA GAGGGAACAT ACTTAGTTAC AACCTTGGAG GGATGGGATG
TTCGGCTGGA ATCATAGCTA TTGATCTTGC TCGTGACATG CTTCAGTCTA ACCCTAATAG TTATGCTGTT
GTTGTGAGTA CTGAGATGGT TGGGTATAAT TGGTACGTGG GAAGTGACAA GTCAATGGTT ATACCTAATT
GTTTCTTTAG GATGGGTTGT TCTGCCGTTA TGCTCTCTAA CCGTCGTCGT GACTTTCGCC ATGCTAAGTA
CCGTCTCGAG CACATTGTCC GAACTCATAA GGCTGCTGAC GACCGTAGCT TCAGGAGTGT GTACCAGGAA
GAAGATGAAC AAGGATTCAA GGGGTTGAAG ATAAGTAGAG ACTTAATGGA AGTTGGAGGT GAAGCTCTCA
AGACAAACAT CACTACCTTA GGTCCTCTTG TCCTACCTTT CTCCGAGCAG CTTCTCTTCT TTGCTGCTTT
GGTCCGCCGA ACATTCTCAC CTGCTGCCAA AACGTCCACA ACCACTTCCT TCTCTACTTC CGCCACCGCA
AAAACCAATG GAATCAAGTC TTCCTCTTCC GATCTGTCCA AGCCATACAT CCCGGACTAC AAGCTCGCCT
TCGAGCATTT TTGCTTCCAC GCGGCAAGCA AAGTAGTGCT TGAAGAGCTT CAAAAGAATC TAGGCTTGAG
TGAAGAGAAT ATGGAGGCTT CTAGGATGAC ACTTCACAGG TTTGGAAACA CTTCTAGCAG TGGAATCTGG
TATGAGTTGG CTTACATGGA GGCCAAGGAA AGTGTTCGTA GAGGCGATAG GGTTTGGCAG ATCGCTTTCG
GTTCTGGTTT TAAGTGTAAC AGTGTGGTGT GGAAGGCAAT GAGGAAGGTG AAGAAGCCAA CCAGGAACAA
TCCTTGGGTG GATTGCATCA ACCGTTACCC TGTGCCTCTC

EL4
FIGURE 9

EL4 protein sequence
Molecular Weight 61953.80 Daltons
550 Amino Acids
71 Strongly Basic(+) Amino Acids (K,R)
58 Strongly Acidic(-) Amino Acids (D,E)
191 Hydrophobic Amino Acids (A,I,L,F,W,V)
147 Polar Amino Acids (N,C,Q,S,T,Y)
9.036 Isolectric Point
14.349 Charge at PH 7.0

MGRSNEQDLL STEIVNRGIE PSGPNAGSPT FSVRVRRRLP DFLQSVNLKY VKLGYHYLIN HAVYLATIPV
LVLVFSAEVG SLSREEIWKK LWDYDLATVI GFFGVFVLTA CVYFMSRPRS VYLIDFACYK PSDEHKVTKE
EFIELARKSG KFDEETLGFK KRILQASGIG DETYVPRSIS SSENITTMKE GREEASTVIF GALDELFEKT
RVKPKDVGVL VVNCSIFNPT PSLSAMVINH YKMRGNILSY NLGGMGCSAG IIAIDLARDM LQSNPNSYAV
VVSTEMVGYN WYVGSDKSMV IPNCFFRMGC SAVMLSNRRR DFRHAKYRLE HIVRTHKAAD DRSFRSVYQE
EDEQGFKGLK ISRDLMEVGG EALKTNITTL GPLVLPFSEQ LLFFAALVRR TFSPAAKTST TTSFSTSATA
KTNGIKSSSS DLSKPYIPDY KLAFEHFCFH AASKVVLEEL QKNLGLSEEN MEASRMTLHR FGNTSSSGIW
YELAYMEAKE SVRRGDRVWQ IAFGSGFKCN SVVWKAMRKV KKPTRNNPWV DCINRYPVPL

EL4
FIGURE 10

EL5 cDNA 1611 bases

TCGAGCTACG TCAGGGCTTT TATATGCACA AATTCTCATA AAGTTTTCAA TTTTATTCCA TTTTTCTCGG
AAGCCATGGA AGCTGCTAAT GAGCCTGTTA ATGGCGGATC CGTACAGATC CGAACAGAGA ACAACGAAAG
ACGAAAGCTT CCTAATTTCT TACAAAGCGT CAACATGAAA TACGTCAAGC TAGGTTATCA TTACCTCATT
ACTCATCTCT TCAAGCTCTG TTTGGTTCCA TTAATGGCGG TTTTAGTCAC AGAGATCTCT CGATTAACAA
CAGACGATCT TTACCAGATT TGGCTTCATC TCCAATACAA TCTCGTTGCT TTCATCTTTC TCTCTGCTTT
AGCTATCTTT GGCTCCACCG TTTACATCAT GAGTCGTCCC AGATCTGTTT ATCTCGTTGA TTACTCTTGT
TATCTTCCTC CGGAGAGTCT TCAGGTTAAG TATCAGAAGT TTATGGATCA TTCTAAGTTG ATTGAAGATT
TCAATGAGTC ATCTTTAGAG TTTCAGAGGA AGATTCTTGA ACGTTCTGGT TTAGGAGAAG AGACTTATCT
CCCTGAAGCT TTACATTGTA TCCCTCCGAG GCCTACGATG ATGGCGGCTC GTGAGGAATC TGAGCAGGTA
ATGTTTGGTG CTCTTGATAA GCTTTTCGAG AATACCAAGA TTAACCCTAG GGATATTGGT GTGTTGGTTG
TGAATTGTAG CTTGTTTAAT CCTACACCTT CGTTGTCAGC TATGATTGTT AACAAGTATA AGCTTAGAGG
GAATGTTAAG AGTTTTAACC TTGGTGGAAT GGGGTGTAGT GCTGGTGTTA TCTCTATCGA TTTAGCTAAA
GATATGTTGC AAGTTCATAG GAATACTTAT GCTGTTGTGG TTAGTACTGA GAACATTACT CAGAATTGGT
ATTTTGGGAA TAAGAAGGCT ATGTTGATTC CGAATTGTTT GTTTCGTGTT GGTGGTTCGG CGATTTTGTT
GTCGAACAAG GGGAAAGATC GTAGACGGTC TAAGTATAAG CTTGTTCATA CCGTTAGGAC TCATAAAGGA
GCTGTTGAGA AGGCTTTCAA CTGTGTTTAC CAAGAGCAAG ATGATAATGG GAAGACCGGG GTTTCGTTGT
CGAAAGATCT TATGGCTATA GCTGGGGAAG CTCTTAAGGC GAATATCACT ACTTTAGGTC CTTTGGTTCT
TCCTATAAGT GAGCAGATTC TGTTTTTCAT GACTTTGGTT ACGAAGAAAC TGTTTAACTC GAAGCTGAAG
CCGTATATTC CGGATTTCAA GCTTGCGTTT GATCATTTCT GTATCCATGC TGGTGGTAGA GCTGTGATTG
ATGAGCTTGA GAAGAATCTG CAGCTTTCGC AGACTCATGT CGAGGCATCC AGAATGACAC TGCACAGATT
TGGAAACACT TCTTCGAGCT CGATTTGGTA TGAACTGGCT TACATAGAGG CTAAAGGTAG GATGAAGAAA
GGAAACCGGG TTTGGCAGAT TGCTTTTGGA AGTGGGTTTA AGTGTAACAG TGCAGTTTGG GTGGCTCTAA
ACAATGTCAA GCCTTCGGTT AGTAGTCCGT GGGAACACTG CATCGACCGA TATCCGGTTA AGCTCGACTT
C

EL5
FIGURE 11

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SUBSTITUTE SHEET (RULE 26)

EL5 protein sequence
Molecular Weight 60874.60 Daltons
537 Amino Acids
63 Strongly Basic(+) Amino Acids (K,R)
47 Strongly Acidic(-) Amino Acids (D,E)
198 Hydrophobic Amino Acids (A,I,L,F,W,V)
148 Polar Amino Acids (N,C,Q,S,T,Y)
9.107 Isolectric Point
17.930 Charge at PH 7.0

SSYVRAFICT NSHKVFNFIP FFSEAMEAAN EPVNGGSVQI RTENNERRKL PNFLQSVNMK YVKLGYHYLI
THLFKLCLVP LMAVLVTEIS RLTTDDLYQI WLHLQYNLVA FIFLSALAIF GSTVYIMSRP RSVYLVDYSC
YLPPESLQVK YQKFMDHSKL IEDFNESSLE FQRKILERSG LGEETYLPEA LHCIPPRPTM MAAREESEQV
MFGALDKLFE NTKINPRDIG VLVVNCSLFN PTPSLSAMIV NKYKLRGNVK SFNLGGMGCS AGVISIDLAK
DMLQVHRNTY AVVVSTENIT QNWYFGNKKA MLIPNCLFRV GGSAILLSNK GKDRRRSKYK LVHTVRTHKG
AVEKAFNCVY QEQDDNGKTG VSLSKDLMAI AGEALKANIT TLGPLVLPIS EQILFFMTLV TKKLFNSKLK
PYIPDFKLAF DHFCIHAGGR AVIDELEKNL QLSQTHVEAS RMTLHRFGNT SSSSIWYELA YIEAKGRMKK
GNRVWQIAFG SGFKCNSAVW VALNNVKPSV SSPWEHCIDR YPVKLDF

EL5
FIGURE 12

EL6 1502 bases

TCTCCGACGATGCCTCAGGCACCGATGCCAGAGTTCTCTAGCTCGGTGAAGCTCAAGTACGTGAAACTTGGTTACCAA
TATTTGGTTAACCATTTCTTGAGTTTTCTTTTGATCCCGATCATGGCTATTGTCGCCGTTGAGCTTCTTCGGATGGGT
CCTGAAGAGATCCTTAATGTTTGGAATTCACTCCAGTTTGACCTAGTTCAGGTTCTATGTTCTTCCTTCTTTGTCATC
TTCATCTCCACTGTTTACTTCATGTCCAAGCCACGCACCATCTACCTCGTTGACTATTCTTGTTACAAGCCACCTGTC
ACGTGTCGTGTCCCCTTCGCAACTTTCATGGAACACTCTCGTTTGATCCTCAAGGACAAGCCTAAGAGCGTCGAGTTC
CAAATGAGAATCCTTGAACGTTCTGGCCTCGGTGAGGAGACTTGTCTCCCTCCGGCTATTCATTATATTCCTCCCACA
CCAACCATGGACGCGGCTAGAAGCGAGGCTCAGATGGTTATCTTCGAGGCCATGGACGATCTTTTCAAGAAAACCGGT
CTTAAACCTAAAGACGTCGACATCCTTATCGTCAACTGCTCTCTTTTCTCTCCCACACCATCGCTCTCAGCTATGGTC
ATCAACAAATATAAGCTTAGGAGTAATATCAAGAGCTTCAATCTTTCGGGGATGGGCTGCAGCGCGGGCCTGATCTCA
GTTGATCTAGCCCGCGACTTGCTCCAAGTTCATCCCAATTCAAATGCAATCATCGTCAGCACGGAGATCATAACGCCT
AATTACTATCAAGGCAACGAGAGAGCCATGTTGTTACCCAATTGTCTCTTCCGCATGGGTGCGGCAGCCATACACATG
TCAAACCGCCGGTCTGACCGGTGGCGAGCCAAATACAAGCTTTCCCACCTCGTCCGGACACACCGTGGCGCTGACGAC
AAGTCTTTCTACTGTGTCTACGAACAGGAAGACAAAGAAGGACACGTTGGCATCAACTTGTCCAAAGATCTCATGGCC
ATCGCCGGTGAAGCCCTCAAGGCAAACATCACCACAATAGGTCCTTTGGTCCTACCGGCGTCAGAACAACTTCTCTTC
CTCACGTCCCTAATCGGACGTAAAATCTTCAACCCGAAATGGAAACCATACATACCGGATTTCAAGCTGGCCTTCGAA
CACTTTTGCATTCACGCAGGAGGCAGAGCGGTGATCGACGAGCTCCAAAAGAATCTACAACTATCAGGAGAACACGTT
GAGGCCTCAAGAATGACACTACATCGTTTTGGTAACACGTCATCTTCATCGTTATGGTACGAGCTTAGCTACATCGAG
TCTAAAGGGAGAATGAGGAGAGGCGATCGCGTTTGGCAAATCGCGTTTGGGAGTGGTTTCAAGTGTAACTCTGCCGTG
TGGAAGTGTAACCGTACGATTAAGACACCTAAGGACGGACCATGGTCCGATTGTATCGACCGTTACCCTGTCTTTATT
CCCGAAGTTGTCAAACTCTA

EL6
FIGURE 13

EL6 protein sequence
Molecular Weight 56687.90 Daltons
500 Amino Acids
59 Strongly Basic(+) Amino Acids (K,R)
46 Strongly Acidic(-) Amino Acids (D,E)
182 Hydrophobic Amino Acids (A,I,L,F,W,V)
127 Polar Amino Acids (N,C,Q,S,T,Y)
8.909 Isolectric Point
14.567 Charge at PH 7.0

SPTMPQAPMP EFSSSVKLKY VKLGYQYLVN HFLSFLLIPI MAIVAVELLR MGPEEILNVW NSLQFDLVQV
LCSSFFVIFI STVYFMSKPR TIYLVDYSCY KPPVTCRVPF ATFMEHSRLI LKDKPKSVEF QMRILERSGL
GEETCLPPAI HYIPPTPTMD AARSEAQMVI FEAMDDLFKK TGLKPKDVDI LIVNCSLFSP TPSLSAMVIN
KYKLRSNIKS FNLSGMGCSA GLISVDLARD LLQVHPNSNA IIVSTEIITP NYYQGNERAM LLPNCLFRMG
AAAIHMSNRR SDRWRAKYKL SHLVRTHRGA DDKSFYCVYE QEDKEGHVGI NLSKDLMAIA GEALKANITT
IGPLVLPASE QLLFLTSLIG RKIFNPKWKP YIPDFKLAFE HFCIHAGGRA VIDELQKNLQ LSGEHVEASR
MTLHRFGNTS SSSLWYELSY IESKGRMRRG DRVWQIAFGS GFKCNSAVWK CNRTIKTPKD GPWSDCIDRY
PVFIPEVVKL

EL6
FIGURE 14

EL7 1548 bases

ATGGACGGTGCCGGAGAATCACGACTCGGTGGTGATGGTGGTGGTGATGGTTCTGTTGGAGTTCAGATCCGACAAACA
CGGATGCTACCGGATTTTCTCCAGAGCGTGAATCTCAAGTATGTGAAATTAGGTTACCATTACTTAATCTCAAATCTC
TTGACTCTCTGTTTATTCCCTCTCGCCGTTGTTATCTCCGTCGAAGCCTCTCAGATGAACCCAGATGATCTCAAACAG
CTCTGGATCCATCTACAATACAATCTGGTTAGTATCATCATCTGTTCAGCGATTCTAGTCTTCGGGTTAACGGTTTAT
GTTATGACCCGACCTAGACCCGTTTACTTGGTTGATTTCTCTTGTTATCTCCCACCTGATCATCTCAAAGCtCCTTAC
GCTCGGTTCATGGAACATTCTAGACTCACCGGAGATTTCGATGACTCTGCTCTCGAGTTTCAACGCAAGATCCTTGAG
CGTTCTGGTTTAGGGGAAGACACTTATGTCCCTGAAGCTATGCATTATGTTCCACCGAGAATTTCAATGGCTGCTGCT
AGAGAAGAAGCTGAACAAGTCATGTTTGGTGCTTTAGATAACCTTTTCGCTAACACTAATGTGAAACCAAAGGATATT
GGAATCCTTGTTGTGAATTGTAGTCTCTTTAATCCAACTCCTTCGTTATCTGCAATGATTGTGAACAAGTATAAGCTT
AGAGGTAACATTAGAAGCTACAATCTAGGCGGTATGGGTTGCAGCGCGGGAGTTATCGCTGTGGATCTTGCTAAAGAC
ATGTTGTTGGTACATAGGAACACTTATGCGGTTGTTGTTTCTACTGAGAACATTACTCAGAATTGGTATTTTGGTAAC
AAGAAATCGATGTTGATACCGAACTGCTTGTTTCGAGTTGGTGGCTCTGCGGTTTTGCTATCGAACAAGTCGAGGGAC
AAGAGACGGTCTAAGTACAGGCTTGTACATGTAGTCAGGACTCACCGTGGAGCAGATGATAAAGCTTTCCGTTGTGTT
TATCAAGAGCAGGATGATACAGGGAGAACCGGGGTTTCGTTGTCGAAAGATCTAATGGCGATTGCAGGGGAAACTCTC
AAAACCAATATCACTACATTGGGTCCTCTTGTTCTACCGATAAGTGAGCAGATTCTCTTCTTTATGACTCTAGTTGTG
AAGAAGCTCTTTAACGGTAAAGTGAAACCGTATATCCCGGATTTCAAACTTGCTTTCGAGCATTTCTGTATCCATGCT
GGTGGAAGAGCTGTGATCGATGAGTTAGAGAAGAATCTGCAGCTTTCACCAGTTCATGTCGAGGCTTCGAGGATGACT
CTTCATCGATTTGGTAACACATCTTCGAGCTCCATTTGGTATGAATTGGCTTACATTGAAGCGAAGGGAAGGATGCGA
AGAGGTAATCGTGTTTGGCAAATCGCGTTCGGAAGTGGATTTAAATGTAATAGCGCGATTTGGGAAGCATTAAGGCAT
GTGAAACCTTCGAACAACAGTCCTTGGGAAGATTGTATTGACAAGTATCCGGTAACTTTAAGTTAT

EL7
FIGURE 15

EL7 protein sequence
Molecular Weight 57848.80 Daltons
516 Amino Acids
59 Strongly Basic(+) Amino Acids (K,R)
48 Strongly Acidic(-) Amino Acids (D,E)
189 Hydrophobic Amino Acids (A,I,L,F,W,V)
131 Polar Amino Acids (N,C,Q,S,T,Y)
8.872 Isolectric Point
12.792 Charge at PH 7.0

MDGAGESRLG GDGGGDGSVG VQIRQTRMLP DFLQSVNLKY VKLGYHYLIS NLLTLCLFPL AVVISVEASQ
MNPDDLKQLW IHLQYNLVSI IICSAILVFG LTVYVMTRPR PVYLVDFSCY LPPDHLKAPY ARFMEHSRLT
GDFDDSALEF QRKILERSGL GEDTYVPEAM HYVPPRISMA AAREEAEQVM FGALDNLFAN TNVKPKDIGI
LVVNCSLFNP TPSLSAMIVN KYKLRGNIRS YNLGGMGCSA GVIAVDLAKD MLLVHRNTYA VVVSTENITQ
NWYFGNKKSM LIPNCLFRVG GSAVLLSNKS RDKRRSKYRL VHVVRTHRGA DDKAFRCVYQ EQDDTGRTGV
SLSKDLMAIA GETLKTNITT LGPLVLPISE QILFFMTLVV KKLFNGKVKP YIPDFKLAFE HFCIHAGGRA
VIDELEKNLQ LSPVHVEASR MTLHRFGNTS SSSIWYELAY IEAKGRMRRG NRVWQIAFGS GFKCNSAIWE
ALRHVKPSNN SPWEDCIDKY PVTLSY

EL7
FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11384

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01H 5/00, C07H 21/00; C12N 15/00, 15/82
US CL :800/205; 435/172.3; 536/23.6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/205; 435/172.3; 536/23.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	WO 95/15387 A2 (CALGENE INC.) 08 June 1995, especially pages 57-71.	1,9,10,18-20,22-26, 28-30 ---------- 2-8,11-17,21,27
X - Y	WO 96/13582 A2 (DNA PLANT TECHNOLOGY CORP.) 09 May 1996, especially pages 33-38.	1,9,10,18,19,24,25 ---------- 2-8,11-17,20-23,26-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 AUGUST 1998	Date of mailing of the international search report 29 SEP 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence for ELIZABETH MCELWAIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11384

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	JAMES et al. Directed Tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) Gene with the Maize Transposon Activator. The Plant Cell. March 1995, Vol. 7, pages 309-319, see especially pages 316-317.	1,9,10 ---------- 2-8,11,17-30